

2

Myocardial Regeneration: Which Cell and Why

ELMOSTAFA EL FAHIME AND JACQUES P. TREMBLAY*

1. Background

During the development, growth of the heart is generally characterized by division of cardiac cells (cardiomyocytes) during the embryonic stages of life, followed after birth by entry into a post-mitotic state. Therefore, growth of the heart after normal development and in cases of cardiac diseases requires enlargement of individual cardiomyocytes (hypertrophy) rather than proliferation of post-mitotic cardiac myocytes.

Cardiovascular disease, including hypertensive diseases and myocardial infarction, leads to loss of cardiac tissue through death of the cells by apoptosis and necrosis. The remaining myocytes are unable to reconstitute the lost tissue, and the diseased heart deteriorates functionally with time. Current therapeutic approaches suffer from limitations and are primarily focused at limiting disease progression rather than repair and restoration of healthy tissue and function. The limited efficacy and co-morbidity of these current treatments have increased the interest to investigate other options, alternative and additional long-term therapeutic approaches.

In this perspective, cell transplantation therapy (CTT) seems to be a potential new therapeutic strategy to achieve cardiac repair.

2. Rational

Preliminary experiments with cardiac tissue established that minced adult newt ventricular tissue could reorganize into a contractile mass when attached to the apex of an injured heart.¹ Subsequent investigations in rats indicated that minced fetal atrial tissue could form stable, contractile grafts in ectopic skeletal muscle beds.² A series of breakthroughs in the last few

*Elmostafa El fahime and Jacques Tremblay, Unité de Génétique humaine, Centre de recherche CHUL (CR-CHUL), 2705 boulevard Laurier, Ste-Foy, G1V 4G2, Québec, Canada

years demonstrated that dispersed preparations of cardiac myocytes were stable when transplanted onto donor mouse hearts.³ Moreover, formation of cell-to-cell contacts, complete with gap junction proteins has been reported in the fetal cardiomyocytes grafts.⁴ More recent work to larger animal models have made a successful transition to clinical trial and are being considered to treat human patients.^{5,6}

3. Potential Cell Types for Heart Repair

Recent work focused on identifying suitable sources of cells for cardiac repair. Potential cells for autologous cell transplantation might be cardiomyocytes,⁷ myoblasts grown from skeletal muscle,⁸ smooth muscle cells from blood vessels,⁹ or hematopoietic or mesenchymal stem cells either mobilized with pharmaceuticals or by biopsy from the bone marrow.^{10,11} In this chapter, we have reviewed the recent literature on the remodeling of the infarcted myocardium with various cell types and discussed the promises and challenges of the cell transplantation therapy for heart diseases (Figure 1).

3.1. *Cardiomyocytes*

It seems logical that cardiac myocytes (cardiomyocytes) would be the best cell type to repair a myocardial infarct. However, successful cardiomyocyte engraftment depends on a complex series of parameters, including terminal differentiation of the transplanted cells and proper excitation/contraction coupling with the host myocardium. Since adult cardiomyocytes do not survive well or proliferate *in vitro*, cardiomyocytes from fetal sources have to be used in cell transplantation experiments. Initial studies generated significant excitement after they demonstrated that cardiomyocytes from fetal mice formed viable grafts after injection into normal myocardium of syngeneic hosts. Furthermore, electron microscopy of engrafted nonimmortalized fetal cardiac myocytes demonstrated that the transplanted cells could form intercalated disks and tight junctions connecting them to the host myocardium.³ Moreover, cardiomyocytes can be proliferated easily in culture and could thus be genetically modified *in vitro*. However, fetal cardiomyocytes are highly sensitive to ischemic injury, and their therapeutic use might ultimately require additional interventions (for example, treatment with cardioprotective genes or drugs).¹² Moreover, human fetal cardiomyocytes are difficult to obtain because of the limited availability of aborted embryos and are limited with respect to their ability to be amplified in culture. Since the embryonic cardiomyocytes are necessarily allogeneic they do not survive in the host heart without adequate immunosuppression. In addition, the use of fetal cardiomyocytes may also face ethical and political difficulties in human application.¹³ This problem accelerated the search of alternative cell types for the repair of the heart.

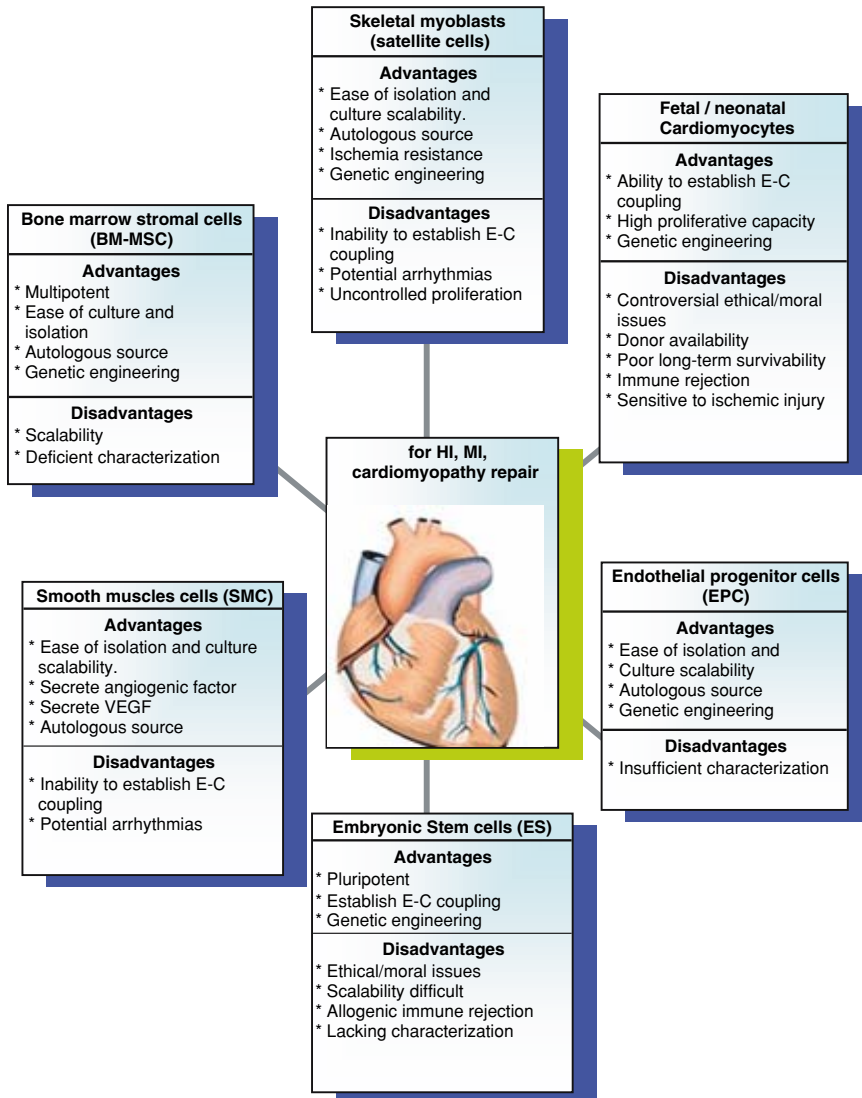


FIGURE 1. Potential cell types for cell-based therapies for the heart (E-C: electro-mechanical coupling; EPC: Endothelial progenitor cells; MI: myocardial infarction; HF: heart failure).

3.2. Myoblasts

Myoblasts or satellite cells are precursor cells attached to skeletal muscle fibers. Every myofiber is intimately associated with a number of satellite cells that lie beneath the basal lamina, closely applied to the plasmalemma. In normal muscles, satellite cells are mitotically quiescent, but become activated to

divide in response to signals released following damage or in response to increased workload or following tissue dissociation (*in vitro*) in culture. After division, satellite cell progeny, termed myoblasts, undergo terminal differentiation and become incorporated into mature muscle fibers as post-mitotic myonuclei reviewed by Bischoff and Heintz.¹⁴ Satellite cells are therefore a population of precursors that provide a reserve capacity to replace differentiated, post-mitotic cells required for the functions of adult skeletal muscles.

Researchers and clinicians took advantage of this natural ability to develop strategies aimed at forming skeletal muscle tissue *in vivo* in various normal or pathological conditions. For example, we have developed a cell therapy approach based on myoblast transplantation to treat Duchenne Muscular Dystrophy patients.¹⁵⁻¹⁹

Skeletal myoblasts exhibit many desirable qualities as donor cells for the treatment of cardiovascular diseases: (1) the ability to be amplified in large quantities and in an undifferentiated state *in vitro*, (2) the possibility of genetically engineering these cells, (3) the capacity to remain viable in ischemic tissue, (4) the potential to maintain their proliferation capacity *in vivo*, and (5) the possibility to achieve autologous transplantation. Successful engraftment of autologous skeletal myoblasts into injured myocardium has been reported in multiple animal models of cardiac injury.²⁰⁻²³ These studies have demonstrated survival and engraftment of myoblasts into infarcted or necrotic hearts, differentiation of the myoblasts into striated cells within the damaged myocardium and improved myocardial functional performance.

On the basis of these preliminary results and the well-established capacity to amplify primary myoblasts from humans, the potential use of skeletal myoblast grafts for treating heart disease has generated considerable interest and clinical trials have begun both in Europe and in the United States.^{24,8} In these studies, the authors report the first intramyocardial transplantation of autologous skeletal myoblasts in a patient with severe ischemic cardiac failure. The encouraging results after an 8-month follow-up underline the potential of this new approach.

However, the transplantation of myoblasts in the heart has some disadvantages. The main one is that these cells do not form intercalated disks and electrical synapses with the host cardiomyocytes. Thus they cannot contract synchronously with the rest of the heart compartment. Moreover, there is conflicting evidence on whether these cells can survive for a long period in the host heart.²⁵ In fact, the myoblasts could produce their therapeutic benefits by secreting transiently growth factor that may prevent the atrophy of the heart wall after an infarct.²⁶ Thus, since the myoblasts do not form new functional cardiac tissue, they are not the best cells for an ideal tissue engineering of the heart.

3.3. Endothelial Cell

The demonstration of the conversion of mouse and human endothelial cells (EC) into cardiomyocytes in *in vitro* co-cultures and *in vivo*²⁷ opened the possibility of using human endothelial cells for cardiac repair. Cell contacts between

the endothelial cells and existing cardiomyocytes seem to be indispensable for such conversion. However, the plasticity of endothelial cells is reduced during development, and the fact that relatively well-differentiated endothelial cells derived from human umbilical veins did form cardiomyocytes,²⁷ made the umbilical cord a possible source of human endothelial cells for therapeutic purposes. Alternatively it might be possible to expand populations of circulating human endothelial progenitor cells (EPC).²⁸ These blood-born cells are thought to originate from a common precursor in adult bone marrow.²⁹ For a review, see Rafii and Lyden.³⁰ They express endothelial lineage markers (i.e., CD34+, Flk-1+, VE-cadherin, PECAM-1, von Willebrand factor, eNOS, and E-selectin) and can be expanded and genetically modified *ex vivo* to yield sufficient numbers for therapeutic applications.^{31,32,28} However, these endothelial cells will have to be further characterized before they can be used for heart therapies.

3.4. *Bone Marrow–Derived Mesenchymal Stem Cells (BM-MSCs)*

BM-MSCs have myogenic potential and are therefore promising candidates for cell-based therapies for myocardial diseases.^{33–36,11} These cells can be isolated on the basis of their adhesive properties, they can be proliferated extensively in culture and thus be easily genetically modified.^{37–40} Moreover, they exhibit a remarkable plasticity.³⁴ Indeed it is now well-established that these cells can differentiate into functional cardiomyocytes under specific culture conditions.^{33,34,41,42} BM-MSCs can be induced to differentiate into synchronously beating cardiomyocytes *in vitro* after treatment of primary cultures of mouse bone marrow with the cytosine analog 5-azacytidine.^{42,43} They are thus an interesting source of autologous cells for cardiac repair. In the light of these reports, systemic administration of BM-MSCs to repair infarcted myocardium has been proposed as an attractive clinical strategy.^{44,36} Altogether these studies indicated that bone marrow progenitors share trans-differentiation into the various cell types required for regeneration and maintenance of the myocardium.⁴⁵ These encouraging preclinical results have led to several recent small-scale feasibility and safety studies to evaluate the therapeutic potential of bone marrow cell transplantation in treatment of ischemic heart diseases and myocardial infarctions.^{46–50}

Nevertheless, these results should be considered preliminary. The nature of the mobilizing, migration and homing signals for bone marrow progenitor cells and the mechanism of differentiation and incorporation into the target tissues need to be identified.

3.5. *Smooth Muscle Cells*

Smooth muscle cells (SMCs) have intrinsic characteristics that may be clinically important in the context of cell therapies for heart failure. Indeed, SMCs have the capacity to divide and to secrete angiogenic factors, such as

nitric oxide (NO),⁵¹ fibroblast growth factors,⁵² and vascular endothelial cell growth factor (VEGF).⁵³ The secretion of these factors may permit to eliminate intractable angina unresponsive to coronary artery bypass and restore contractility to hibernating cardiomyocytes. Li et al. showed that fetal smooth muscle cells can be successfully transplanted into myocardial scar tissue to form smooth muscle tissue, to stimulate angiogenesis, to limit remodelling and to improve myocardial function.⁹ The consequences of transplanting the smooth muscle cells in the heart have to be further investigated before a clinical trial of such transplantation is undertaken.

3.6. *Embryonic Stem Cells*

It is well-established that totipotent murine embryonic stem (ES) cells can give rise to a variety of cell lineages *in vitro* and *in vivo* including cardiac myocytes. Pure cultures of cardiomyocytes with expanding capacity in culture and therefore suitable for transplantation have been obtained by simple genetic selection protocols.⁵⁴ The transplanted cells formed intra-cardiac grafts that were stable up to 7 weeks. Given the excellent potential demonstrated by mouse ES cells, much effort has been spent on the development of human ES cell lines. Kehat et al. described the generation of a reproducible cardiomyocyte differentiation system from human ES cells.⁵⁵ The generated myocytes were shown to display functional and structural properties consistent with early-stage cardiomyocytes.

However, it is very important to keep in mind the existence of striking differences between the human and murine stem cell models. Indeed, human ES cells have a very low efficiency of conversion into cardiomyocytes compared with those of mice, a slower time course of differentiation, and only a lower number of human ES cells are able to undergo differentiation and spontaneous contraction.⁵⁵

Although, the development of the human ES cell technology holds great potential for the field of myocardial regeneration, a number of issues will need to be met before any clinical applications can be expected. First of all, the use of embryonic cell lines is still controversial in some countries. There are also many technical issues. Some of the milestones that need to be achieved include: (1) Development of strategies for directing differentiation of the human ES cells into the cardiac lineage; (2) Selection protocols should be devised to allow generation of pure population of cardiomyocytes for transplantation; (3) The differentiation process should be up-scaled to yield clinically relevant number of cells for transplantation; (4) Several technical and conceptual issues regarding *in vivo* cell transplantation should be resolved and (5) Methods for circumventing immune rejection of these allogeneic cells should be developed. In fact, approaches aimed at reduction of the mass of alloreactive T cells are being developed and these and other novel therapies with particular relevance to the anticipated immune response mounted against ES-derived cell transplants will probably be used.⁵⁶ These

strategies may also include establishing 'banks' of major histocompatibility complex antigen-typed human ES cells, genetically manipulating ES cells to suppress the immune response, such as by knocking-out the major histocompatibility complexes⁵⁷ and possibly also by nuclear transfer techniques (therapeutic cloning).⁵⁸

4. Conclusion

Treatment of damaged myocardium after myocardial infarction by cell transplantation is becoming an increasingly promising therapeutic approach. Ideally, the donor cells should be amplified efficiently in culture and would lead to regeneration of infarcted myocardial tissue, including cardiogenic differentiation with local angiogenesis. Two of the most widely used cell types for cardiac repair today are skeletal muscle-derived progenitors, or myoblasts, and bone marrow-derived progenitors. Both cell types share advantages over other cells used for cardiac repair (or at least for limiting infarcts) in that they are readily available, autologous, exhibit a high proliferative potential *in vitro* and share a low potential for tumor genesis.

However, the transplantation of autologous cells to repair the heart also has serious drawbacks. It is labor intensive since isolation and cell proliferation has to be done for each patient. This procedure also delays the treatment. The 'ideal' cell to treat the heart should be transplantable without delay to any patient without a sustained immunosuppression. Such ideal cells may be obtained one day by the genetic engineering of embryonic stem cells.

Through cellular therapies, the concept of "growing" heart muscle and vascular tissue and manipulating the myocardial cellular environment may revolutionize the approach to treating heart disease.

5. References

1. Bader, D., and Oberpriller, J. O. 1978. Repair and reorganization of minced cardiac muscle in the adult newt (*Notophthalmus viridescens*). *J Morphol* 155: 349.
2. Jockusch, H., Fuchtbauer, E. M., Fuchtbauer, A., Leger, J. J., Leger, J., Maldonado, C. A., and Forssmann, W. G. 1986. Long-term expression of isomyosins and myoendocrine functions in ectopic grafts of atrial tissue. *Proc Natl Acad Sci U S A* 83: 7325.
3. Soonpaa, M. H., Koh, G. Y., Klug, M. G., and Field, L. J. 1994. Formation of nascent intercalated disks between grafted fetal cardiomyocytes and host myocardium. *Science* 264: 98.
4. Koh, G. Y., Soonpaa, M. H., Klug, M. G., Pride, H. P., Cooper, B. J., Zipes, D. P., and Field, L. J. 1995. Stable fetal cardiomyocyte grafts in the hearts of dystrophic mice and dogs. *J Clin Invest* 96: 2034.
5. Grounds, M. D., White, J. D., Rosenthal, N., and Bogoyevitch, M. A. 2002. The role of stem cells in skeletal and cardiac muscle repair. *J Histochem Cytochem* 50: 589.

6. Melo, L. G., Pachori, A. S., Kong, D., Gneccchi, M., Wang, K., Pratt, R. E., and Dzau, V. J. 2004. Gene and cell-based therapies for heart disease. *Faseb J* 18: 648.29.
7. Soonpaa, M. H., Daud, A. I., Koh, G. Y., Klug, M. G., Kim, K. K., Wang, H., and Field, L. J. 1995. Potential approaches for myocardial regeneration. *Ann N Y Acad Sci* 752: 446.
8. Menasche, P., Hagege, A. A., Scorsin, M., Pouzet, B., Desnos, M., Duboc, D., Schwartz, K., Vilquin, J. T., and Marolleau, J. P. 2001. Myoblast transplantation for heart failure. *Lancet* 357: 279.
9. Li, R. K., Jia, Z. Q., Weisel, R. D., Merante, F., and Mickle, D. A. 1999. Smooth muscle cell transplantation into myocardial scar tissue improves heart function. *J Mol Cell Cardiol* 31: 513.
10. Kocher, A. A., Schuster, M. D., Szabolcs, M. J., Takuma, S., Burkhoff, D., Wang, J., Homma, S., Edwards, N. M., and Itescu, S. 2001. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med* 7: 430.
11. Orlic, D., Kajstura, J., Chimenti, S., Limana, F., Jakoniuk, I., Quaini, F., Nadal-Ginard, B., Bodine, D. M., Leri, A., and Anversa, P. 2001b. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci U S A* 98: 10344.
12. Reinecke, H., Zhang, M., Bartosek, T., and Murry, C. E. 1999. Survival, integration, and differentiation of cardiomyocyte grafts: a study in normal and injured rat hearts. *Circulation* 100: 193.
13. Reinlib, L., and Field, L. 2000. Cell transplantation as future therapy for cardiovascular disease?: A workshop of the National Heart, Lung, and Blood Institute. *Circulation* 101: E182.
14. Bischoff, R., and Heintz, C. 1994. Enhancement of skeletal muscle regeneration. *Dev Dyn* 201: 41.
15. Skuk, D., Roy, B., Goulet, M., Chapdelaine, P., Bouchard, J. P., Roy, R., Dugre, F. J., Lachance, J. G., Deschenes, L., Helene, S., Sylvain, M., and Tremblay, J. P. 2004. Dystrophin expression in myofibers of Duchenne muscular dystrophy patients following intramuscular injections of normal myogenic cells. *Mol Ther* 9: 475.
16. Skuk, D., and Tremblay, J. P. 2003a. Cell therapies for inherited myopathies. *Curr Opin Rheumatol* 15: 723.
17. Skuk, D., and Tremblay, J. P. 2003b. Myoblast transplantation: the current status of a potential therapeutic tool for myopathies. *J Muscle Res Cell Motil* 24: 285.
18. Tremblay, D. S. a. J. P. 2001. "Engineering" Myoblast Transplantation. *Graft* 4: 558.
19. Tremblay, J. P., Malouin, F., Roy, R., Huard, J., Bouchard, J. P., Satoh, A., and Richards, C. L. 1993. Results of a triple blind clinical study of myoblast transplantations without immunosuppressive treatment in young boys with Duchenne muscular dystrophy. *Cell Transplant* 2: 99.
20. Atkins, B. Z., Lewis, C. W., Kraus, W. E., Hutcheson, K. A., Glower, D. D., and Taylor, D. A. 1999. Intracardiac transplantation of skeletal myoblasts yields two populations of striated cells in situ. *Ann Thorac Surg* 67: 124.
21. Dorfman, J., Duong, M., Zibaitis, A., Pelletier, M. P., Shum-Tim, D., Li, C., and Chiu, R. C. 1998. Myocardial tissue engineering with autologous myoblast implantation. *J Thorac Cardiovasc Surg* 116: 744.
22. Murry, C. E., Wiseman, R. W., Schwartz, S. M., and Hauschka, S. D. 1996. Skeletal myoblast transplantation for repair of myocardial necrosis. *J Clin Invest* 98: 2512.

23. Taylor, D. A., Atkins, B. Z., Hungspreugs, P., Jones, T. R., Reedy, M. C., Hutcheson, K. A., Glower, D. D., and Kraus, W. E. 1998. Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nat Med* 4: 929.
24. Dib, N., Diethrich, E. B., Campbell, A., Goodwin, N., Robinson, B., Gilbert, J., Hobohm, D. W., and Taylor, D. A. 2002. Endoventricular transplantation of allogenic skeletal myoblasts in a porcine model of myocardial infarction. *J Endovasc Ther* 9: 313.
25. Suzuki, K., Smolenski, R. T., Jayakumar, J., Murtuza, B., Brand, N. J., and Yacoub, M. H. 2000. Heat shock treatment enhances graft cell survival in skeletal myoblast transplantation to the heart. *Circulation* 102: III216.
26. Menasche, P. 2002. [Cell therapy: myoblast autograft]. *Bull Acad Natl Med* 186: 73.
27. Condorelli, G., Borello, U., De Angelis, L., Latronico, M., Sirabella, D., Coletta, M., Galli, R., Balconi, G., Follenzi, A., Frati, G., Cusella De Angelis, M. G., Gioglio, L., Amuchastegui, S., Adorini, L., Naldini, L., Vescovi, A., Dejana, E., and Cossu, G. 2001. Cardiomyocytes induce endothelial cells to trans-differentiate into cardiac muscle: implications for myocardium regeneration. *Proc Natl Acad Sci U S A* 98: 10733.
28. Kawamoto, A., Gwon, H. C., Iwaguro, H., Yamaguchi, J. I., Uchida, S., Masuda, H., Silver, M., Ma, H., Kearney, M., Isner, J. M., and Asahara, T. 2001. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. *Circulation* 103: 634.
29. Asahara, T., Masuda, H., Takahashi, T., Kalka, C., Pastore, C., Silver, M., Kearne, M., Magner, M., and Isner, J. M. 1999. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 85: 221.
30. Rafii, S., and Lyden, D. 2003. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. *Nat Med* 9: 702.
31. Iwaguro, H., Yamaguchi, J., Kalka, C., Murasawa, S., Masuda, H., Hayashi, S., Silver, M., Li, T., Isner, J. M., and Asahara, T. 2002. Endothelial progenitor cell vascular endothelial growth factor gene transfer for vascular regeneration. *Circulation* 105: 732.
32. Kalka, C., Masuda, H., Takahashi, T., Kalka-Moll, W. M., Silver, M., Kearney, M., Li, T., Isner, J. M., and Asahara, T. 2000. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. *Proc Natl Acad Sci U S A* 97: 3422.
33. Jackson, K. A., Majka, S. M., Wang, H., Pocius, J., Hartley, C. J., Majesky, M. W., Entman, M. L., Michael, L. H., Hirschi, K. K., and Goodell, M. A. 2001. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest* 107: 1395.
34. Jiang, Y., Jahagirdar, B. N., Reinhardt, R. L., Schwartz, R. E., Keene, C. D., Ortiz-Gonzalez, X. R., Reyes, M., Lenvik, T., Lund, T., Blackstad, M., Du, J., Aldrich, S., Lisberg, A., Low, W. C., Largaespada, D. A., and Verfaillie, C. M. 2002. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 418: 41.
35. Orlic, D., Kajstura, J., Chimenti, S., Jakoniuk, I., Anderson, S. M., Li, B., Pickel, J., McKay, R., Nadal-Ginard, B., Bodine, D. M., Leri, A., and Anversa, P. 2001a. Bone marrow cells regenerate infarcted myocardium. *Nature* 410: 701.
36. Toma, C., Pittenger, M. F., Cahill, K. S., Byrne, B. J., and Kessler, P. D. 2002. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* 105: 93.

37. Blau, H. M., Brazelton, T. R., and Weimann, J. M. 2001. The evolving concept of a stem cell: entity or function? *Cell* 105: 829.
38. Gao, J., Dennis, J. E., Muzic, R. F., Lundberg, M., and Caplan, A. I. 2001. The dynamic *in vivo* distribution of bone marrow-derived mesenchymal stem cells after infusion. *Cells Tissues Organs* 169: 12.
39. Pittenger, M. F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Douglas, R., Mosca, J. D., Moorman, M. A., Simonetti, D. W., Craig, S., and Marshak, D. R. 1999. Multilineage potential of adult human mesenchymal stem cells. *Science* 284: 143.
40. Wakitani, S., Saito, T., and Caplan, A. I. 1995. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. *Muscle Nerve* 18: 1417.
41. Hakuno, D., Fukuda, K., Makino, S., Konishi, F., Tomita, Y., Manabe, T., Suzuki, Y., Umezawa, A., and Ogawa, S. 2002. Bone marrow-derived regenerated cardiomyocytes (CMG Cells) express functional adrenergic and muscarinic receptors. *Circulation* 105: 380.
42. Makino, S., Fukuda, K., Miyoshi, S., Konishi, F., Kodama, H., Pan, J., Sano, M., Takahashi, T., Hori, S., Abe, H., Hata, J., Umezawa, A., and Ogawa, S. 1999. Cardiomyocytes can be generated from marrow stromal cells *in vitro*. *J Clin Invest* 103: 697.
43. Tomita, S., Li, R. K., Weisel, R. D., Mickle, D. A., Kim, E. J., Sakai, T., and Jia, Z. Q. 1999. Autologous transplantation of bone marrow cells improves damaged heart function. *Circulation* 100: II247.
44. Orlic, D., Hill, J. M., and Arai, A. E. 2002. Stem cells for myocardial regeneration. *Circ Res* 91: 1092.
45. Kamihata, H., Matsubara, H., Nishiue, T., Fujiyama, S., Tsutsumi, Y., Ozono, R., Masaki, H., Mori, Y., Iba, O., Tateishi, E., Kosaki, A., Shintani, S., Murohara, T., Imaizumi, T., and Iwasaka, T. 2001. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation* 104: 1046.
46. Assmus, B., Schachinger, V., Teupe, C., Britten, M., Lehmann, R., Dobert, N., Grunwald, F., Aicher, A., Urbich, C., Martin, H., Hoelzer, D., Dimmeler, S., and Zeiher, A. M. 2002. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation* 106: 3009.
47. Perin, E. C., Dohmann, H. F., Borojevic, R., Silva, S. A., Sousa, A. L., Mesquita, C. T., Rossi, M. I., Carvalho, A. C., Dutra, H. S., Dohmann, H. J., Silva, G. V., Belem, L., Vivacqua, R., Rangel, F. O., Esporcatte, R., Geng, Y. J., Vaughn, W. K., Assad, J. A., Mesquita, E. T., and Willerson, J. T. 2003. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation* 107: 2294.
48. Stamm, C., Westphal, B., Kleine, H. D., Petzsch, M., Kittner, C., Klinge, H., Schumichen, C., Nienaber, C. A., Freund, M., and Steinhoff, G. 2003. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet* 361: 45.
49. Strauer, B. E., Brehm, M., Zeus, T., Kostering, M., Hernandez, A., Sorg, R. V., Kogler, G., and Wernet, P. 2002. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 106: 1913.
50. Tse, H. F., Kwong, Y. L., Chan, J. K., Lo, G., Ho, C. L., and Lau, C. P. 2003. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. *Lancet* 361: 47.

51. Koide, M., Y. Kawahara, et al. (1993). "Cyclic AMP-elevating agents Induce an inducible type of nitric oxide synthase in cultured vascular smooth muscle cells. Synergism with the induction elicited by inflammatory cytokines." *J Biol Chem* 268(33): 24959-66.
52. Ali, N. and D. K. Agrawal (1994). "Guanine nucleotide binding regulatory proteins: their characteristics and identification." *J Pharmacol Toxicol Methods* 32(4): 187-96.
53. Stavri, G. T., I. C. Zachary, et al. (1995). "Basic fibroblast growth factor upregulates the expression of vascular endothelial growth factor in vascular smooth muscle cells. Synergistic interaction with hypoxia." *Circulation* 92(1): 11-4.
54. Klug, M. G., Soonpaa, M. H., Koh, G. Y., and Field, L. J. 1996. Genetically selected cardiomyocytes from differentiating embryonic stem cells form stable intracardiac grafts. *J Clin Invest* 98: 216.
55. Kehat, I., Kenyagin-Karsenti, D., Snir, M., Segev, H., Amit, M., Gepstein, A., Livne, E., Binah, O., Itskovitz-Eldor, J., and Gepstein, L. 2001. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest* 108: 407.
56. Bradley, J. A., Bolton, E. M., and Pedersen, R. A. 2002. Stem cell medicine encounters the immune system. *Nat Rev Immunol* 2: 859.
57. Grusby, M. J., Auchincloss, H., Jr., Lee, R., Johnson, R. S., Spencer, J. P., Zijlstra, M., Jaenisch, R., Papaioannou, V. E., and Glimcher, L. H. 1993. Mice lacking major histocompatibility complex class I and class II molecules. *Proc Natl Acad Sci U S A* 90: 3913.
58. Lanza, R. P., Chung, H. Y., Yoo, J. J., Wettstein, P. J., Blackwell, C., Borson, N., Hofmeister, E., Schuch, G., Soker, S., Moraes, C. T., West, M. D., and Atala, A. 2002. Generation of histocompatible tissues using nuclear transplantation. *Nat Biotechnol* 20: 689.

Stem Cell Therapy and Tissue Engineering for
Cardiovascular Repair

From Basic Research to Clinical Applications

Dib, N.; Taylor, D.A.; Diethrich, E.B. (Eds.)

2006, XIX, 335 p., Hardcover

ISBN: 978-0-387-25788-4