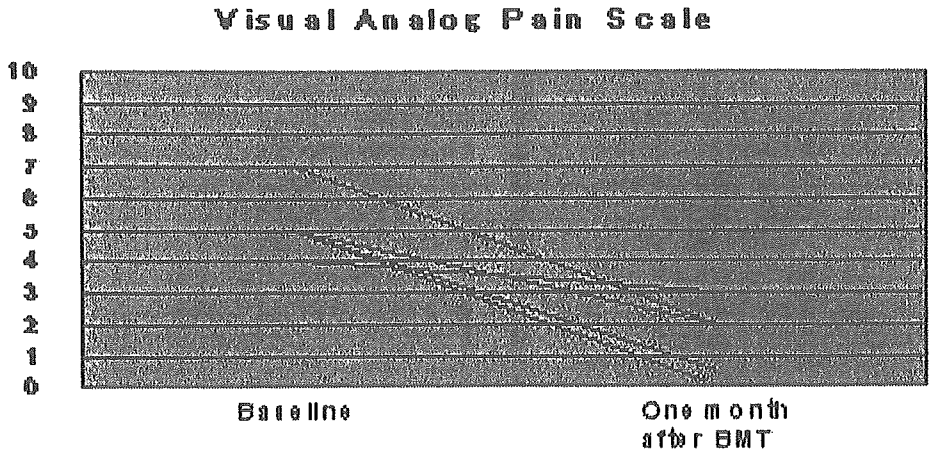


Fig. 2. The Visual analog pain scale in all patients.

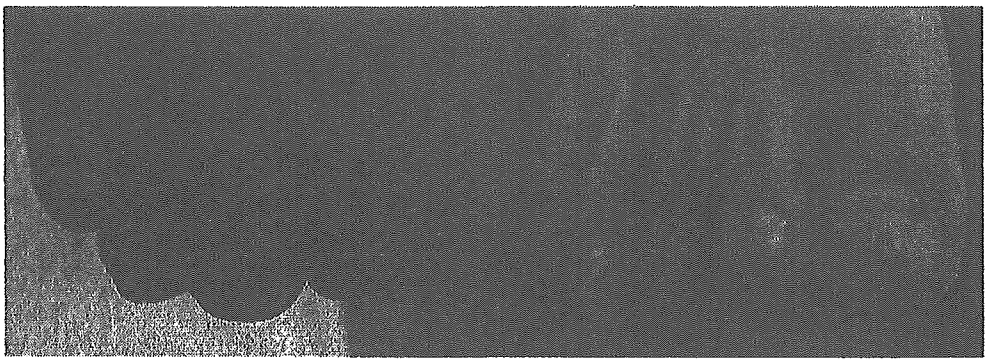
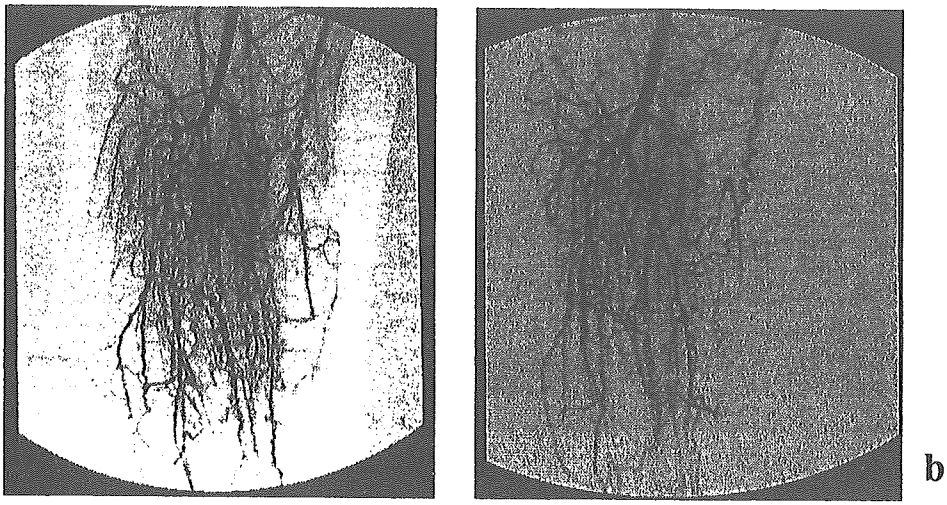
a  b

Fig. 3a, b. The skin ulcers in a patient before a and one month after autologous bone marrow transplantation b.

Conventional angiography was performed before and one month after BMT, but there was no significant changes in any of the patients (Fig.4).

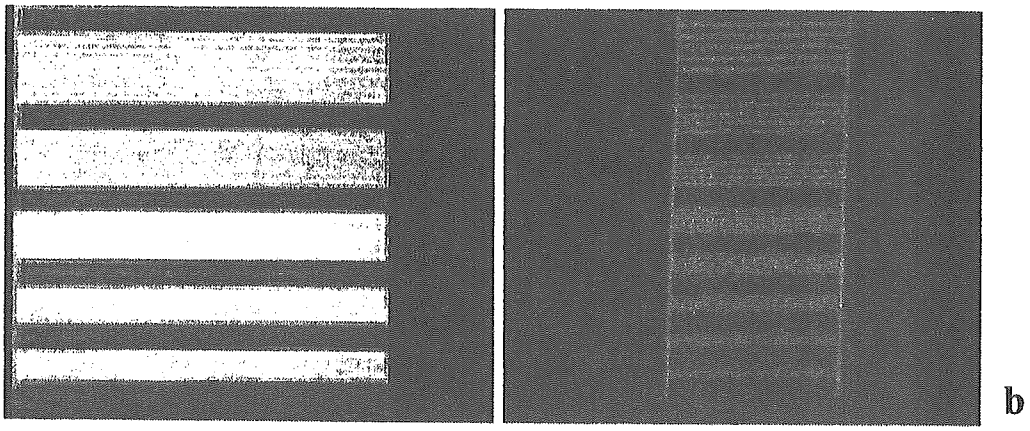


a b

Fig. 4a, b. The conventional angiographic findings in the patient before a and one month after autologous bone marrow transplantation b.

Novel micro-angiography

The novel micro-angiography can detect to within a limit 50 of μm, although a detection limit of a conventional angiography is 250μm (Fig.5).



a b

Fig. 5a, b. The detection limits on a conventional angiography a and the novel micro-angiography b using a line chart

Collateral micro-vessels, which were 100-500μm or less in diameter, were demonstrated more clearly in micro-angiography than conventional angiography (Fig.6).

The sum of radio-absorptions at the point of 1m distance from the X-ray source in clinical settings was 300 mSv. for 10 seconds.

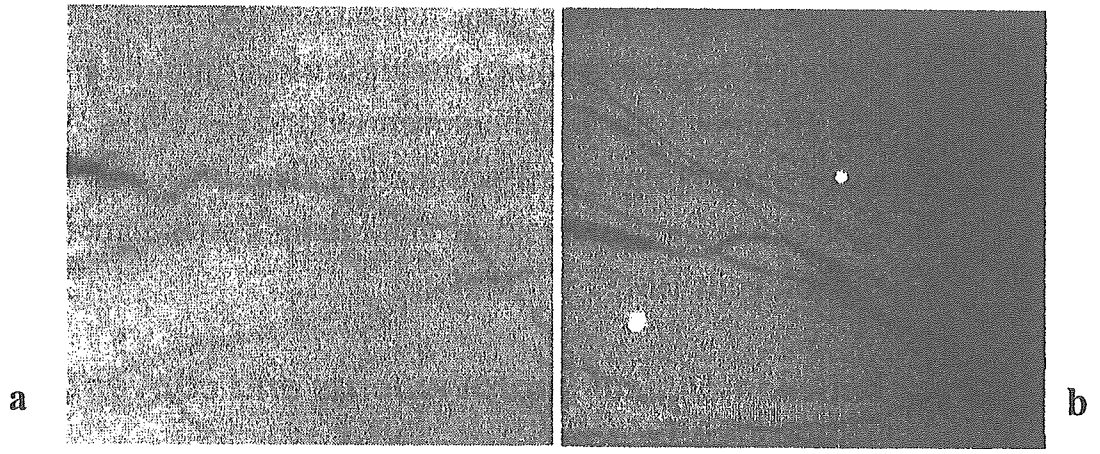


Fig. 6a, b. In 2.5x2.5cm view size, Collateral micro-vessels after therapeutic angiogenesis in the rabbit limb ischemia model. Vessel sizes in the range of 100-500μm or less, were demonstrated in the novel micro-angiography b more clearly than in a conventional angiography a. The diameter of the line in the micro-angiography is 130μm.

Discussion

Autologous BMT improved chronic severe limb ischemia due to Bue rger's disease. Conventional angiography could not disclose develope d collateral vessels after BMT. A novel micro-angiography technique could illuminate promoted collateral vessels after therapeutic angioge nesis in rabbit models although a conventional angiography did not. The sum of radio-absorptions in the novel angiography could be acc-epted in clinical settings.

Autologous BMT and Buerger's disease

Bone marrow harvests need an amount of more than 500ml bone marrow fluid and general anesthesia in therapeutic angiogenesis using BMT. Such factors have practical limitations to select candidates with peripheral artery disease complicated with systemic atherosclerosis and aging for BMT. Buerger's disease is a segmental vasculitis that affects the distal arteries of the upper and lower extremities. It typically occurs in young people. The majority of patients with Buerger's disease have pain at rest and digital

ulcerations and are hard to treat by revasculaizations, including catheter angioplasty and surgical bypass grafting, because of peripheral artery lesions. Patients with Buerger's disease, however, tend to have less systemic atherosclerotic lesions and normal cardiac function. These suggest that patients with Buerger's disease are the ideal candidates for therapeutic angiogenesis using autologous BMT.

Discrepancy between clinical improvements and conventional angiographic findings after BMT

BMT improved critical limb ischemia clinically. Promoted collateral vessels after the treatment were not, however, visualized well by conventional angiography. These vessels are quite small and the detection limit of small vessels by conventional angiography is about 200μm in diameter.

Novel micro-angiography

Recently, synchrotron radiation system characterized by high brightness, monochromatic and collimated nature bypass, revealed micro-vessels in situ. However the high cost of a synchrotron system strictly limits its clinical application (100 million dollars or more). We developed an in-house micro-angiographic system with a relatively low cost of approximately 1million dollars, which consisted of a high-voltage power X-ray source and a detecting system with a high spatial resolution (25μm) and high sensitivity (100 times of CCD camera). We evaluated collateral micro-vessels one month after therapeutic angiogenesis by using the conventional and micro-angiographic system. The in-house micro-vessel angiographic system could detect the micro-vessels more precisely than conventional angiographic system. We thought that the present micro-angiography should be useful for evaluating efficacy of therapeutic angiogenesis in clinical settings.

Conclusions

Conventional angiography failed to disclose the promoted collateral vessels after BMT although BMT improved the critical limb ischemia clini-

cally. The in-house micro-angiographic system could detect the micro-vessels more precisely than conventional angiographic system and the sum of the radio-absorption in the equipment could be acceptable in clinical settings. The novel in-house micro-angiographic system can be useful in the evaluation of therapeutic angiogenesis clinically.

References

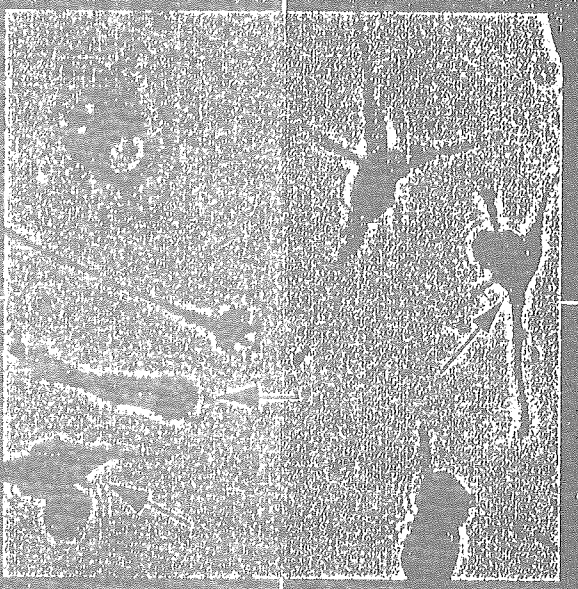
Asahara T, Murohara T, Sullivan A, et al.(1997) Isolation of Putative Progenitor Endothelial Cells for Angiogenesis. Science 275:964-966

Iba O, Matsubara H, Nozawa Y, et al.(2002) Angiogenesis by Implantation of Peripheral Blood Mononuclear Cells and Platelets into Ischemic Limbs. Circulation 106:2019-2025

Inaba S, Egashira K, Komori K (2002) Peripheral-blood or bone-marrow mononuclear cells for therapeutic angiogenesis? Lancet 360:2083.

Shintani S, Murohara T, Ikeda H, et al.(2001) Augmentation of Postnatal Neovascularizaion with Autologous Bone Marrow Transplantation. Circulation 103: 897-903

Tateishi-Yuyama E, Matsubara H, Murohara T, et al.(2002) Therapeutic angiogenesis for patients with imb ischemia by autologous transplantation of bone-marrow cells:a pilot study and a randomised controlled trial. Lancet,360:427-35



H. Mori • H. Matsuda (Eds.)

Cardiovascular Regeneration Therapies Using Tissue Engineering Approaches

Hidezo Mori, M.D., Ph.D.
Director, Department of Cardiac Physiology
National Cardiovascular Center Research Institute
5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan

Hikaru Matsuda, M.D., Ph.D.
Professor, Division of Cardiovascular Surgery, Department of Surgery
Osaka University Graduate School of Medicine
2-2-E1 Yamadaoka, Suita, Osaka 565-0871, Japan

Library of Congress Control Number: 2004117651

ISBN 4-431-23925-1 Springer-Verlag Tokyo Berlin Heidelberg New York

Springer is a part of Springer Science+Business Media
springeronline.com

Printed in Japan

Typesetting: Camera-ready by the editors
Printing and binding: Nikkei Printing, Japan

Printed on acid-free paper

Regeneration of Myocardium Using Bone Marrow Cells

Shinji Tomita[1], Takeshi Nakatani[2]

[1]Cardiothoracic Surgical Unit, Auckland City Hospital, Auckland, New Zealand [2] Department of Organ Transplantation, National Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka, 565-8565, Japan

Summary. Bone marrow cells are advantageous for exogenous cell transplantation to treat end-stage heart failure regard to autologous source, no ethical issue, capacity to regenerate myocardium, induction of angiogenesis. Based on basic research showing regenerating myocardium using bone marrow, clinical trials in several places were conducted like gold rush in recent years. Endogenous-stem cell therapy may be also a promising strategy. Self-renewal of myocardium may be partly derived from bone marrow and the myocardium itself, which was thought to be a terminally differentiated organ. The past new technologies have been developed and their use expanded despite a lack of concrete evidence regarding their effectiveness. However, we still have a lot of unanswered questions including optimal cell population, cell density, and exact mechanism responsible for the improvement of cardiac dysfunction, fate of fusioned cells, cardiac environmental factors, regulation of proliferation and differentiation of transplanted cells, efficient cell tracking method in human. People involved in this field must be careful as they proceed, as inappropriately designed research might ruin the future of the field of regenerative medicine. Cell-based therapy will continue to expand at a rapid rate over the next decade. Whether the benefits of cell-based therapy are evident in the future remains to be seen.

Key Word. Heart, Bone marrow cells, Cell-based therapy, Exogenous-stem cell, Endogenous-stem cell

Introduction

Once the heart is damaged, some cardiomyocytes will necrose and residual cardiomyocytes may compensate heart function with hypertrophy. However, heart failure may eventually develop in the end stage. Although heart transplantation is an effective treatment for end-stage heart failure, the shortage of donors is a major limiting factor. (Hosenpud JD, et al. 1998). While heart transplantation currently remains the best choice, other alternatives, such as mechanical support and drug therapy, are being investigated. In our hope exogenously transplanted cells may compensate loss of cardiomyocytes to improve the damaged heart function. Since then a variety of cell-types have been investigated enthusiastically (Li RK, et al. 1999, Li RK, et al. 1996, Taylor DA, et al. 1998).

Exogenous cell transplantation

Bone marrow cells have many advantages compared to other cell sources. The technique of bone marrow aspiration is an established procedure in hematology. There are no immunological or ethical issues because of the autologous source. Bone marrow comprises two major systems; one haematopoietic and the other mesenchymal. Bone, cartilage, and fat derive from "mesenchymal stem cells". However, the lack of a universal way to identify "mesenchymal stem cells" represents a major obstacle.

In 1999, Makino S, et al. (Makino S, et al. 1999) and our group (Tomita S, et al. 1999) reported that cardiomyocytes could be generated from 5-azacytidine-treated bone marrow cells. Bittner et al reported that intravenously transplanted bone marrow cells could also differentiate into cardiomyocytes (Bittner RE, et al. 1999).

Many groups have tried to purify adult stem cells of several phenotypes, including cardiomyocytes, endothelial cells, and smooth muscle cells. Orlic et al. injected Lin-C-kit+cells to regenerate the infarcted myocardium (Orlic D, et al. 2001). Jackson et al. used side population (SP) cells (Jackson KA, et al. 2001). More recently, Beltrami AP, et al. reported Lin-C-kit+cells with the properties of cardiac stem cells. When injected into an ischemic heart, these cells reconstitute the myocardium including new ves-

sels and myocytes (Beltrami AP, et al. 2003). In contrast, Murry CE, et al. (Murry CE, et al. 2004) and Balsam LB, et al. (Balsam LB, et al. 2004) demonstrated that haematopoietic stem cells (Lin-c-kit+, Lin-c-kit+Sca+) did not transdifferentiate into cardiomyocytes in myocardial infarcts using cardiomyocyte-restricted and ubiquitously expressed reporter transgenes. Researchers are attempting to manipulate stem cells in vitro and in vivo. However, currently nobody has established the best way to expand human cardiomyocytes or cardiomyoblasts to a number that would be sufficient for clinical application.

Historically allograft cells were used for research in early the 1990's because of easiness; however, those cells were eliminated due to immunorejection despite cyclosporine therapy (Li RK, et al. 1997). Therefore, many researchers recently have utilized autologous or syngenic animal model to test applicability to clinical setting. However, if we can utilize allograft or xenogeneic cells without immunorejection, we will be able to expand cell-based therapy much more efficiently and industrially. There are several reports showing the possibility of immunotolerance or no immunorejection using bone marrow cells. Liechty KW, et al. transplanted human mesenchymal stem cells into sheep. They observed human cells inside of sheep without immunorejection (Liechty KW, et al. 2000). Chiu's group highlighted the possibility of xenonenic bone marrow stromal cells without rejection (Saito T, et al. 2002). Embryonal stem cell (ES cell) research may be an alternative in the future. In contrast to adult stem cells it is easier to expand ES cells in vitro. They provide a good tool to investigate the mechanism of cardiac differentiation at the genetic level. However ethical and immunological issues provide major hurdles that need to be overcome before pursuing clinical applications.

Based on animal data Menasche's group in France conducted the first clinical trial using skeletal myoblast. They showed that possible efficacy but faced on a critical issue: arrhythmia (Menasche P, et al. 2003). Four out of 10 patients had ventricular tachycardia and required an Automatic Intracardiac Defibrillator (AICD). They speculated that formation of gap junctions between host and donor cells might cause arrhythmia. The AICDs were not activated due to arrhythmia after implantation. In further trials, some institutions may implant AICDs prophylactically before cell

transplantation. The interpretation of this reported adverse effect of myoblast transplantation is hampered by the differing methods of cell culture used in different institutions. Standardization of the methods employed for cell culture by Good Manufactural Practice (GMP) is essential for the analysis and reproducibility of outcomes. However it is difficult to generate guidelines and regulations due to the rapid development occurring in this field. So far, there are a lot of human and animal studies using bone marrow cells, which did not detect any harmful effect regarding arrhythmia (Fujii H, et al. 2004). This issue should be investigated more deeply in further studies.

A number of studies from Japan have contributed to the regenerative medicine field. Noishiki Y, et al. reported that bone marrow cells seeded PTFE grafts and produced rapid endothelialisation inside the graft (Noishiki Y, et al. 1996). Asahara T, et al. in Boston proposed endothelial progenitor cells (EPC) originating from bone marrow contribute to angiogenesis and vasculogenesis in ischaemic myocardium (Asahara T, et al. 1999, Asahara T, et al. 1997). Murohara's group promoted bone marrow mononuclear cells (BMMNC) instead of EPC (Shintani S, et al. 2001) for their clinical research into ischaemic disease (Tateishi-Yuyama E, et al. 2002). BMMNC do not require special techniques for culture in contrast to myoblasts and EPC. Autologous donation avoids problems related to ethics and immune rejection. There is some evidence that transplanted BMMNC contribute to revascularization. Many people believe that cytokines released by bone marrow cells play a major role in angiogenesis (Tateishi-Yuyama E, et al. 2002). These benefits have conferred a significant advantage to bone marrow cells over other cell types and resulted in rapid expansion of this method into clinical application from Japan (Hamano K, et al. 2002) to other countries (Tse HF, et al. 2003). Their main concept is to rescue the ischemic heart through the mechanism of vasculogenesis and angiogenesis by bone marrow cells.

Based on easiness to utilize bone marrow cells and the hypothesis that bone marrow cells can regenerate the heart, many clinical trials have been conducted in recent years, too (Assmus B, et al. 2002, Strauer BE, et al. 2001). They are trying to merge the two concepts: one is for angiogenesis and the other is for myogenesis. Because we are not sure what exact

mechanism to improve regarding the impaired function of the heart, even in animal studies, we do not know what the major contributor is in human.

Endogenous-stem cell therapy

For many years people believed that adult cardiomyocytes cannot proliferate and regenerate themselves. In 2001 Anversa's group reported that even adult cardiomyocytes are capable of proliferation and self regeneration in the diseased heart and opened the door to endogenous-stem-cell therapy (Beltrami AP, et al. 2001). Other groups have also reported regeneration of cardiomyocytes in varying amounts (Laflamme MA, et al. 2002). These studies have indicated the existence of endogenous-stem cells which can contribute to the self-renewal of myocardium. In contrast to exogenous cell therapy methods, endogenous therapy does not require cell culture or surgical techniques.

In 2001, Orlic D, et al. reported that granulocyte colony-stimulating factor (GCSF) and stem-cell-factor (SCF) improved heart function and the survival rate after myocardial infarction (Orlic D, et al. 2001). We confirmed that bone marrow was a source of regenerated cardiomyocytes using a GFP chimera mouse infarction (Fukuhara S, et al. 2002) and doxorubicin model (Tomita S, et al. 2004) and that GCSF promoted bone marrow cells to migrate. However, it does not seem that migrated bone marrow cells support pump function directly because of the very small number of bone marrow- derived cardiomyocytes found in the heart (Fukuhara S, et al. in press). GCSF has a variety of biological roles, including effects on healing through the enhancement of proliferation. We hypothesized that GCSF directly affected diseased myocardium and enhanced proliferation of Troponin I positive cells through GCSF-receptor (Hamamoto M, et al. in press). More recently, Beltrami AP, et al. reported cardiac stem cells exist in the heart (Beltrami AP, et al. 2003).

Sata M, et al. reported that hematopoietic stem cells may contribute to atherosclerosis (Sata M, et al. 2002). Recently a Korean group conducted GCSF treatment for patients who had acute myocardial infarction followed by intracoronary stenting. They stopped enrollment because of unexpectedly high rate of in-stent restenosis in those patients who received GCSF

(Kang HJ, et al. 2004). They raised the important point that endogenous-stem cells could migrate into both injured myocardium and atherosclerotic lesions. If we can elucidate the physiological mechanism of endogenous-stem cell migration, we may be better able to control this process in the future treatment of myocardial injury.

Cardiac environmental Factors

Several reports have emphasized the importance of cardiac environmental factors in the cardiac differentiation of stem cells (Liechty KW, et al. 2000, Wang JS, et al. 2000). There are many possible factors, including myocardial injury, paracrine factors from the host, direct interaction between cardiomyocytes and stem cells (Condorelli G, et al. 2001, Reinecke H, et al. 2000, Tomita S, et al. 2002), intramyocardial pressure, and electrical stimulation.

We reported that direct cell-cell interaction is a key mechanism in the differentiation of bone marrow stromal cells derived from transgenic mice expressing green fluorescent protein (GFP mouse) into cardiac lineage in a co-culture system (Fukuhara S, et al. 2003). Bone marrow stromal cells showed synchronous contraction with cardiomyocytes from day 2. Immunostaining showed myosin-heavy chain from day 1, connexin 43 and atria-nucleotide peptide from day 2, and troponin I from day 4. Studies have demonstrated the ability of human circulating bone marrow cells to differentiate into cardiomyocytes (Badorff C, et al. 2003). In 2002, a critical issue of cell fusion was reported (Terada N, et al. 2002). In this study, embryonic stem cells and bone marrow cells were derived from a GFP mouse. A proliferating colony expressing GFP had DNA from the embryonic stem cells. Because of the small rate of fusion, this phenomenon could not be fully explained. The co-cultivation of human stem cells with cells from other animal species cannot proceed until the possibility of trans-species gene transfer is completely eliminated. Several reports investigated this fusion issue, however, those results were scattered (Balsam LB, et al. 2004, Murry CE, et al. 2004). Oh H, et al. reported that adult heart derived cardiac progenitor cells expressing stem cell antigen-1 differentiate into cardiomyocytes roughly equally, with and without fusion to

host cells (Oh H, et al. 2003). If the fusion occurs as a physiological process, it would be worth to investigate the fate and the role of fusioned cells in vivo.

Many unresolved issues remain in the progress towards future clinical applications of cell-based therapies. Unanswered questions include optimal cell population, cell density, and mechanism of effect responsible for the improvement of cardiac dysfunction. If we can resolve these issues then we might be able to match certain disease processes to the best cell therapy for treatment.

As progress continues, adverse effects will be identified. Arrhythmia may be a critical issue. In addition, we still do not know how the proliferation and differentiation of transplanted cells are regulated and the effects of environment. So far there have been no reports in humans of malignant tumor formation or ectopic differentiation such as bone formation. Although there are many reports supporting cell transplantation using animals, we still do not know how transplanted cells behave in humans. No efficient cell tracking method has been developed.

Whether the benefits of cell-based therapy or/and those of gene therapy or angiogenic protein therapy in the future remains to be seen by well-designed study.

Reference

Asahara T, Masuda H, Takahashi T, Kaka C, Pastore C, Silver M, Kearne M, Magner M, Isner JM (1999) Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 85(3):221-228

Asahara T, Murohara T, Sullivan A, Silver M, van der ZR, Li T, Witzenbichler B, Schatteman G, Isner, JM (1997) Isolation of putative progenitor endothelial cells for angiogenesis. Science 275(5302):964-967

Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, Grunwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S, Zeiher AM. (2002) Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). Circulation Dec 10; 106(24):3009-17

Badorff C, Brandes RP, Popp R, Rupp S, Urbich C, Aicher A, et al.(2003) Trans-differentiation of blood-derived human adult endothelial progenitor cells into functionally active cardiomyocytes. Circulation 107(7):1024-32

Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. (2004) Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature 428(6983):668-73

Beltrami AP, Urbanek K, Kajstura J, Yan SM, Finato N. Bussani R, Nadal-Ginard B, Silvestri F, Leri A, Beltrami CA, Anversa P (2001) Evidence that human cardiac myocytes divide after myocardial infarction. N Engl J Med 344(23): 1750-1757

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.(2003) Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 114(6):763-76

Bittner R E, Schofer C, Weipoltshammer K, Ivanova S, Streubel B, Hauser E, Freilinger M, Hoger H., Elbe-Burger A, Wachtler F (1999) Recruitment of bone-marrow-derived cells by skeletal and cardiac muscle in adult dystrophic mdx mice. Anat Embryol (Berl) 199(5): 391-396

Fujii H, Tomita S, Nakatani T, Fukuhara S, Hanatani A, Ohtsu Y, Ishida M, Yutani C, Miyatake K, Kitamura S (2004) A Novel application of myocardial contrast echocardiography to evaluate angiogenesis by autologous bone marrow cell transplantation in chronic ischemic pig model. J Am Coll Cardiol 43(7):1299-1305

Condorelli G, Borello U, De Angelis L, Latronico M, Sirabella D, Coletta M, Galli R, Balconi G, Follenzi A, Frati G, Cusella De Angelis MG, Gioglio L, Amuchastegui S, Adorini L, Naldini L, Vescovi A, Dejana E, Cossu G (2001) Cardiomyocytes induce endothelial cells to trans-differentiate into cardiac muscle: implications for myocardium regeneration. Proc Natl Acad Sci USA 98(19):10733-38

Fukuhara S, Tomita S, Ohtsu Y, Ishida M, Yutani C, Kitamura S, Nakatani T (2002) G-CSF Promoted Bone Marrow Cells to Migrate into Infarcted Heart and Differentiate into Cardiomyocytes. Circulation 106[suppl II]:A1870

Fukuhara S, Tomita S, Nakatani T, Yamashiro S, Morisaki T, Yutani C, Kitamura S (2003) Direct cell-to-cell interaction of cardiomyocytes is a key for bone

marrow stromal cells to go into cardiac lineage in vitro. J Thorac Cardivasc Surg 125:1470-1480

Fukuhara S, Tomita S, Nakatani T, Yutani C, Kitamura S (2004) Endogenous bone marrow-derived stem cells reconstituted myocardium only in the small proportion after acute myocardial infarction. J Heart Lung Transplant (in press)

Hamamoto M, Tomita S, Nakatani T, Yutani C, Yamashiro S, Sueda T, Yagihara T, Kitamura S (2004) Granurocyte-colony stimulating factor directly enhances the proliferation of human adult heart cells derived from idiopathic dilated cardiomyopathy. J Heart Lung Transplant (in press)

Hamano K, Li TS, Kobayashi T, Hirata K, Yano M, Kohno M, Matsuzaki M (2002) Therapeutic angiogenesis induced by local autologous bone marrow cell implantation. Ann Thorac Surg 73(4):1210-15

Hosenpud JD, Bennett LE, Keck BM, Fiol B, Boucek MM, Novick RJ (1998) The Registry of the International Society for Heart and Lung Transplantation: fifteenth official report-1998. J Heart Lung Transplant 17(7):656-668

Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman M L, Michael LH, Hirschi KK, Goodell MA (2001) Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 107(11):1395-1402.

Kang HJ, Kim HS, Zhang SY, Park KW, Cho HJ, Koo BK, Kim YJ, Soo Lee D, Sohn DW, Han KS, Oh BH, Lee MM, Park YB. (2004) Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. Lancet 363 (9411):751-756

Laflamme MA, Myerson D, Saffitz JE, Murry CE (2002) Evidence for cardiomyocyte repopulation by extracardiac progenitors in transplanted human hearts. Circ Res 90(6):634-640

Li RK, Jia ZQ, Weisel RD, Merante F, Mickle DA (1999) Smooth muscle cell transplantation into myocardial scar tissue improves heart function. J Mol Cell Cardiol 31(3):513-522

Li RK, Jia ZQ, Weisel RD, Mickle DA, Zhang J, Mohabeer MK, Rao V, Ivanov J (1996) Cardiomyocyte transplantation improves heart function. Ann Thorac Surg. 62(3):654-660

Li RK, Mickle DA, Weisel RD, Mohabeer MK, Zhang J, Rao V, Li G, Merante F, Jia ZQ (1997) Natural history of fetal rat cardiomyocytes transplanted into adult rat myocardial scar tissue. Circulation 96(9 Suppl): II-176-186

Liechty KW, MacKenzie TC, Shaaban AF, Radu A, Moseley AM, Deans R, Marshak DR, Flake AW (2000) Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. Nat Med 6(11):1282-1286

Makino S, Fukuda K, Miyoshi S, Konishi F, Kodama H, Pan J, Sano M, Takahashi T, Hori S, Abe H, Hata J, Umezawa A, Ogawa S (1999) Cardiomyocytes can be generated from marrow stromal cells in vitro. J Clin Invest 103(5): 697-705

Menasche P, Hagege AA, Vilquin JT, Desnos M, Abergel E, Pouzet B, et al. (2003) Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. J Am Coll Cardiol 41(7):1078-83

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.(2004) Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 428(6983):664-8

Noishiki Y, Tomizawa Y, Yamane Y, Matsumoto A (1996) Autocrine angiogenic vascular prosthesis with bone marrow transplantation. Nat Med 2(1):90-93

Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ, Entman ML, Schneider MD. (2003) Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. Proc Natl Acad Sci USA 100(21):12313-8

Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P (2001a) Bone marrow cells regenerate infarcted myocardium. Nature 410(6829):701-705

Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. (2001b). Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci USA 98(18):10344-49

Reinecke H, MacDonald G H, Hauschka SD, Murry CE (2000) Electromechanical coupling between skeletal and cardiac muscle. Implications for infarct repair. J Cell Biol 149(3):731-740

Saito T, Kuang JQ, Bittira B, Al Khaldi A, Chiu RC (2002) Xenotransplant cardiac chimera: immune tolerance of adult stem cells. Ann Thorac Surg 74(1):19-24

Sata M, Saiura A, Kunisato A, Tojo A, Okada S, Tokuhisa T, Hirai H, Makuuchi M, Hirata, Y., and Nagai, R. (2002). Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. Nat Med 8(4):403-409

Shintani S, Murohara T, Ikeda H, Ueno T, Sasaki K, Duan J, Imaizumi T (2001) Augmentation of postnatal neovascularization with autologous bone marrow transplantation. Circulation 103(6):897-903

Strauer BE, Brehm M, Zeus T, Kostering M, Hernandez A, Sorg RV, Kogler G, Wernet P (2002) Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 106(15):1913-1918

Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, Amano K, Kishimoto Y, Yoshimoto K, Akashi H, Shimada K, Iwasaka T, Imaizumi T (2002) Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. Lancet 360(9331):427-435

Taylor DA, Atkins BZ, Hungspreugs P, Jones TR, Reedy MC, Hutcheson KA, Glower DD, Kraus WE (1998) Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. Nat. Med. 4(8):929-933

Terada N, Hamazaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y, Meyer EM, Morel L, Petersen BE, Scott EW (2002) Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature, 416(6880): 542-545

Tomita S, Li RK, Weisel RD, Mickle DA, Kim EJ, Sakai T, Jia ZQ (1999) Autologous transplantation of bone marrow cells improves damaged heart function. Circulation 100(19 Suppl):II247-256

Tomita S, Nakatani T, Fukuhara S, Morisaki T, Yutani C, Kitamura S (2002) Bone marrow stromal cells contract synchronously with cardiomyocytes in a coculture system. Jpn J Thorac Cardiovasc Surg 50(8):321-324

Tomita S, Ishida M, Nakatani T, Fukuhara S, Hisashi Y, Ohtsu Y, Suga M, Yutani C, Yagihara T, Yamada K, Kitamura S (2004) Bone Marrow is a Source of Regenerated Cardiomyocytes in Doxorubicin-Induced- Cardiomyopathy, and

GCSF Enhances Migration of Bone Marrow Cells and Attenuates Cardiotoxicity of Doxorubicin Under Electronmicroscopy. J Heart Lung Transplant 23(5):577-584

Tse H F, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP (2003) Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. Lancet 361(9351):47-49

Wang JS, Shum-Tim D, Galipeau J, Chedrawy E, Eliopoulos N, Chiu RC (2000) Marrow stromal cells for cellular cardiomyoplasty: feasibility and potential clinical advantages. J Thorac Cardiovasc Surg 120(5):999-1006