ISSN : 0974 - 7532 Volume 6 Issue 10

Trade Science Inc.

Research & Reviews in

BioSciences

Review

RRBS, 6(10), 2012 [280-287]

Potential of different types of stem cells for cardiomyocyte regeneration

Pavana Thomas, Sarah Sunitha*, V.Krishna Murthy

Department of Biotechnology, P.E.S Institute of Technology, 100 Feet Ring Road, BSK III Stage, Bangalore-560085, (INDIA)

E -mail : sarahjohn125@gmail.com

Received: 2nd July, 2012 ; Accepted: 18th September, 2012

ABSTRACT

Cardiac disorders are a major cause of death in the world today, causing a greater number of deaths as compared to even communicable diseases like AIDS, tuberculosis, diarrhea and malaria. Cardiac disease may occur due to either atherosclerosis or ischemia, both of which lead to decreased blood supply to the myocardium that would bring about necrosis of cardiac tissue, finally resulting in a decrease in the ejection fraction of the heart. Stem cells with their ability to divide and differentiate into many kinds of cells, help in regenerating the injured tissue in the heart that has lost its ability to relax and contract, thus resulting in an improvement in the overall functioning of the heart. Embryonic stem cells, haematopoietic stem cells, mesenchymal stem cells, induced pluripotent stem cells and resident cardiac stem cells are some of the types of cells capable of cardiac regeneration. This article describes the properties of these stem cells, their advantages, shortcomings and factors that affect their efficacy in cardiac regeneration.

KEYWORDS

Myocardial infarction;
Stem cells;
Differentiation;
Regeneration.

INTRODUCTION

Heart attacks and congestive heart failure continue to be among the world's major health challenges despite much advancement in cardiovascular medicine[1]. The destruction of cardiomyocytes can be a result of hypertension, decrease in the blood supply to the heart muscle caused by coronary artery disease, or a myocardial infarction leading to the sudden closing of the coronary artery causing anoxia. Despite improvements in surgical procedures, drug therapy and organ transplantation, a greater part of the patients with congestive heart failure die within few years of initial diagnosis. Scientists are now exploring ways to save lives by introducing new cells to replace dead or injured cells so that the weakened heart muscle can regain its pumping action. One important type of cell that can be regenerated is the cardiomyocyte, that contracts to eject the blood out of the ventricle. Two other cell types that are important for appropriate functioning of the heart are the vascular endothelial cell, which forms the inner lining of blood vessels and the non striated muscle cell, which forms the wall of blood vessels. Researchers have discovered that under highly specific growth conditions,

stem cells can develop into new cardiomyocytes and vascular endothelial cells in vitro[2]. This ability to provide tissue for the damaged heart may be exploited for human benefit. This approach has enormous advantages over heart transplant, particularly in the light of scarcity of donor hearts available to meet current transplantation needs. Until now, the lack of a suitable human cardiac cell source has been the major hindrance in regenerating the human myocardium, either by cardiac tissue engineering or by cell-based transplantation. Cardiomyocytes grow to be terminally differentiated soon after birth and lose their ability to divide and proliferate[3].

The potential of the following types of stem cells for cardiomyocyte regeneration are examined:

Embryonic stem cells

Embryonic stem cells (ESCs) are procured from the inner cell mass of 5-6 day blastocysts. They have unlimited expansion ability and unrestricted plasticity, offering a reliable source of cells that are restricted to the lineage of cells in need of repair and regeneration[3]. They are regarded as a single source of diverse cell types (endothelial, fibroblasts, cardiac muscle cells) required for cardiovascular regeneration[4]. ESCs are seen as an appropriate source for generation of unlimited quantities of cardiomyocytes[7]. In addition, they are capable of differentiating into other cell types like endothelial cells, thus showing the capability of forming all the required cardiac tissue components apart from cardiomyocytes[5].

ESCs have the ability to produce factors like VEGF, IGF, TNF á, â, IL-1. Collectively, these paracrine factors help in survival, formation of new blood vessels, differentiation, remodeling and contraction[6]. When human ESCs were cultured in- vitro in suspensions, embryoid bodies were formed. These embryoid bodies can differentiate along all the three germ layers. 8.1% of these embryoid bodies were seen to have spontaneously contracting areas. They also stained positively when stained with anti-cardiac myosin heavy chain. RT-PCR showed the expression of numerous cardiac-specific genes and transcription factors[8]. hESC-derived cardiomyocytes were seen to show properties of premature cardiac cells. ESCs are seen to possess the property of transdifferentiation, whereby they can differentiate into particular cell types by reprogramming[9]. This would allow the use of cells from autologous sources thus preventing the risk of rejection of exogenous cells due to a mismatch.

However, there are a few drawbacks of using ESCs as a source of stem cells for cardiac regeneration. Embryoid bodies contain a large number of cell types, making cardiogenesis extremely inefficient. Also, undifferentiated hESC may lead to the spontaneous formation of large, benign masses of haphazardly differentiated tissues called teratomas[10-12]. Ethical issues are a major hurdle in the use of hESC for research and therapy[13]. This is because production of a hESC line involves the destruction of an embryo. Stem cells derived from the embryo are allogenic and carry the risk of rejection when transplanted into the host[14].

It was seen that when ESCs were transplanted into mice after Doxorubicin induced cardiomyopathy, it led to significant decrease in various adverse pathological mechanisms as well as enhancement in cardiac regeneration[15]. In another study, when mice ESCs growing on collagen I/II scaffolds was modified with adhesion peptides RGD (arginine-glycine-aspartic acid), the embryoid bodies were observed to differentiate efficiently into beating cardiomyocytes on collagen scaffolds[16]. The use of microRNAs as modulators in ESCs, was seen to provide enhanced cardiomyocyte differentiation, resulting in enhanced cardiac repair, regeneration and function in mice[17]. SSEA-4, Tra 1-60, Tra-181 and Oct-4 are the prominent ESC markers and on differentiation to cardiac cells, they express cardiac troponin I[18].

Haematopoietic stem cells

Haematopoietic stem cells (HSCs) are located in the bone marrow[13]. There are many sub-populations of cells in the bone marrow, and these need to be sorted based on various markers[14]. Significant cardiac improvements were seen in patients administered with CD34+ bone marrow cells[16]. These are multipotent cells with a promising potential to treat an array of conditions.

HSCs are taken from the patient to whom they must be administered for treatment. Thus, they are considered to be an autologous source of stem cells and can easily cross the immunological barrier, posing no risk of rejection in the host. Unlike hESC, which is fraught by ethical issues, HSCs have no such complications as they

Review

do not involve the destruction of an embryo. HSCs are derived easily from the hip bone of the patient and pose no problems of lack of donors[13]. HSCs exhibit plasticity and can give rise to all types of blood cells, cardiac tissue and blood vessels[19]. This is credited to their capacity for multilineage differentiation and self-renewal[20]. Nevertheless, since HSCs are derived from the patient's own body, there is a time delay between isolation of cells, purification, and consequent selection of adequate number of cardiac progenitors ex-vivo and finally introduction of these cells into the patient.

Cell therapy is seen to be highly effective only when a well defined population of cells is administered[13]. Knowledge of the mechanisms by which stem cells undergo extravasation, followed by homing at the site of cardiac injury is extremely important to optimize therapeutic use of stem cells[19]. Very few of the injected cells are seen to develop into cardiomyocytes[21].

In a placebo controlled phase II study conducted to evaluate the outcome administration of granulocyte colony stimulating factor (G-CSF) on transplanted HSCs, it was seen that early treatment with G-CSF resulted in increased perfusion to the infracted area, which may be due to enhanced neovascularization[24]. In a rabbit model, it was noted that in the initial stages of myocardial ischemia, the increase in cytokines TNFá and VEGF was accompanied by mobilization and consequent homing of HSCs into the infracted myocardium[25]. The various markers that prove differentiation into cardiomyocytes include cardiac á/â myosin heavy chain, sarcomeric myosin heavy chain and cardiac troponinI[26].

Mesenchymal stem cells

Among the various kinds of stem cells used for cardiac function restoration, numerous pre-clinical trials have been performed on mesenchymal stem cells (MSCs), in order to evaluate their effectiveness in regenerating the heart after a myocardial infarction[27]. They exhibit multipotency and are precursors to muscle, bone, tendon, ligaments, adipose tissue and fibroblasts[28,29]. They can be isolated, expanded in the lab to a sufficient quantity and then used for regeneration into cardiomyocytes, smooth muscles and endothelial cells due to their capacity of multi-lineage differentiation[30, 31]. Due to their capacity for quick division in culture, MSCs from one donor may be collected, cultured in-

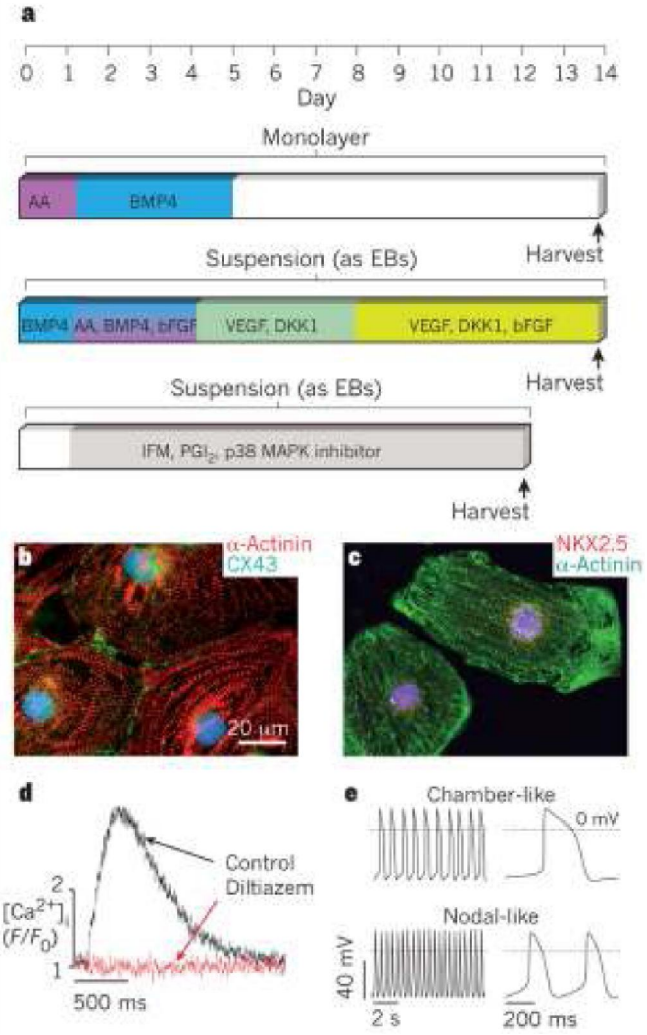

Figure 1 : Representative human ESC-derived cardiomyocytes, differentiated using the monolayer protocol (top timeline in a), immunostained for á-actinin (red) and CX43 (green). Nuclei are shown in blue. Representative human iPSC-derived cardiomyocytes, differentiated using the monolayer protocol (top timeline in a), immunostained for á-actinin (green) and the transcription factor NKX2.5 (red). Intracellular [Ca^{2+}] ([Ca^{2+}]$_i$) transients in a human ESC-derived cardiomyocyte before (black) or after (red) the application of diltiazem, an L-type Ca^{2+}-channel blocker. The absence of [Ca^{2+}] transients after diltiazem treatment indicates that extracellular Ca^{2+} is required to initiate intracellular Ca^{2+} release, just as in adult cardiomyocytes. F/F_0 denotes the change in fluorescence intensity. Human ESC-derived cardiomyocytes show the characteristic action-potential properties of either working chamber (top) or nodal (bottom) cardiomyocytes, indicating early subtype specification[37].

(Reprinted with permission from Macmillan Publishers limited, (Nature International Journal of Weekly Sciences, Heart Regeneration, Michael A. Laflamme et al, Vol 473, Issue 7347, May 2011)

vitro and then used for treatment of numerous patients[29]. MSCs do not express HLA class II normally, and modify T-cell responses.

The regenerative capacity of MSCs decreases with age, thus their effectiveness comes down drastically when used as an autologous source in old patients[29]. Besides, cardiac cell therapy for older patients will require MSCs from young donors for greater renewal capacity and this involves allogenic transplantation[32,33]. Although MSCs do not normally express HLA Class II, recent research has suggested that MSCs may switch immune states in vivo to express HLA Class II and induce an immunogenic response[33,34].

In a recent study, aged MSCs in mice were initially exposed to glucose depletion to enhance age affected function. This resulted in enhanced expression of paracrine factors like IGF-1, FGF-2, VEGF and SDF-1á in hearts transplanted with preconditioned aged MSCs. Therefore it was concluded that pre-conditioning of aged MSCs with glucose depletion can restore the ability of aged cells to repair senescent infarcted myocardium by improving proliferation and delaying senescence[35]. In another study it was investigated whether trimetazidine (TMZ) could improve survival of MSCs in conditions of hypoxia. It was also tested whether TMZ could influence the rates of survival, differentiation, and subsequent activities of transplanted MSCs in rat hearts that had been subjected to acute myocardial infarction (AMI). It was concluded that implantation of MSCs combined with TMZ treatment resulted in up-regulation of anti-apoptotic proteins and was superior to administration of MSCs alone, due to superior MSCs viability and cardiac function recovery[36]. A study conducted to evaluate the effect Tongxinluo (TXL) treatment around transplanted MSCs in swine hearts with acute myocardial infarction (AMI), concluded that TXL treatment resulted in a significant survival and differentiation potential of implanted MSC by inhibition of apoptosis and oxidative stress accompanied by significant benefits in cardiac function[38].

MSC differentiated into cardiomyocytes express markers like Nkx2.5, human atrial natriuretic peptide, myosin light chain-2á and GATA-4[39].

Induced pluripotent stem cells

Somatic cells can be reprogrammed into the pluripotent state by the viral transduction of transcription factors like Oct 3/4, Sox2, Klf4, and c-Myc[40] or by fusion of somatic cells with pluripotent cells[41]. These cells can display pluripotency both in vitro and in vivo, differentiating into cells derived from all three germ layers[40]. Thus, generation of induced pluripotent stem cells (iPSCs) is considered a ground breaking step towards generation of patient-specific pluripotent stem cells for various applications[42].

iPSCs have the capacity to differentiate into atrial, nodal and ventricular cells, with properties similar to the native cardiomyocytes. They are also seen to differentiate into smooth muscles and endothelial cells, supplying the infracted area with sufficient blood, leading to an overall improvement in cardiac functioning[43]. iPSCs are endowed with all the advantages of ESCs, without involving any ethical issues. These can therefore be used as an alternative to ESCs[44].

On the contrary, iPSCs give rise to numerous types of cardiomyocytes. Such a mixed population of cells raises concerns of pro-arrhythmia effects. Moreover, the ability of iPSCs to mature after transplantation is being investigated. Presence of undifferentiated cells may result in the formation of tumors consequent to transplantation[44]. In addition, use of viral vectors for transduction of transcription factors may pose potential problems in the host. These are now being replaced by adenoviruses and plasmid mediated transfections[45,46].

In a study conducted to assess the effect of scar tissue composition on engraftment of iPSCs into infarcted myocardium, a tricell patch (Tri-P) was created with induced pluripotent stem cell-derived cardiomyocytes, endothelial cells and mouse embryonic fibroblasts and affixed over the entire infarcted area in adenylyl cyclase 6 (AC6) overexpressing mice, 7 days after myocardial infarction. Application of a Tri-P in AC6 mice resulted in higher induced pluripotent stem cell engraftment accompanied by angiomyogenesis and improvement in LV function[47]. In another study conducted to determine if the acquired cardiogenicity was affected by nuclear reprogramming, mouse embryonic fibroblasts were reprogrammed with or without c-MYC. It was concluded that nuclear reprogramming independent of c-MYC enhances production of pluripotent stem cells with innate cardiogenic potential[48]. Another study used an excisable polycistronic lentiviral vector to demonstrate proficient derivation of iPS cells

Review

free of exogenous reprogramming transgenes. The presence of the transgenes in the induced pluripotent cells is a matter of concern and this excisable lentivirus vector helps to replace the integrated reprogramming factors[49].

The markers observed on differentiation of iPSCs into cardiomyocytes are Nkx2.5, áMHC, Mlc2v, and cTnT[50].

Cardiac stem cells

Unlike previously believed, the adult heart is seen to have a potential for cardiac regeneration. This is noticed primarily after a myocardial infarction or a pressure overload[51]. This ability is attributed to the resident cardiac stem cells (CSCs) present in the heart. However, this capacity is not sufficient to repair the heart completely after a myocardial infarction[52]. But if these cells are transplanted in a large number exogenously, they are seen to have positive effects in the functioning of the heart after a cardiac arrest.

CSCs are of two types: myogenic CSCs (mCSCs) which give rise to cardiomyocytes and vascular CSCs (vCSCs), which yield endothelial and smooth muscle cells[53,54]. Thus, use of CSCs can give rise to all kinds of cells required to improve overall cardiac function[55]. CSCs are obtained from the heart and are seen to successfully form only cardiac cells, without the formation of teratomas as is the case with ESCs.

However, CSCs are seen to undergo apoptosis due to decreased telomerase activity leading to telomerase shortening. This results in reduced life of the administered CSC leading to deterioration in cardiac functioning[56].

Adult feline hearts, treated with Isoproterenol to induce cardiac injuries were monitored for increase in cellular proliferation by incorporation of 5-Bromodeoxyuridine. During recovery, a significant increase in the number of cells that was positive for 5-Bromodeoxyuridine was observed[57]. CSCs are selected based on expression of the markers c-kit, Sca-1 and MDR-1[58].

CONCLUSION

A comparative study of the different types of stem cells used in cardiac repair has revealed that each category has its pros and cons. ESCs have enormous potential, but they are limited by several ethical issues surrounding its use. This has spurred the discovery of alternate sources of stem cells. HSCs and MSCs are limited by lack of regenerative capacity and controversy over their commitment to cardiac lineage. iPSCs are restricted by the use of viral vectors for reprogramming and CSCs by their reduced telomerase activity.

Stem cell therapy does offer new promise, but it cannot succeed without a thorough understanding of the mechanisms involved, which is achievable only through extensive research, apart from validation through clinical trials. In short, stem cell research has immense potential to unravel novel avenues for regenerative medicine and help in decreasing mortality rates due to cardiovascular diseases.

TABLE 1 : Outcomes of stem cell therapy for cardiac regeneration.

Type of cell	Outcomes	Reference
ESCs	Preserved LV dimensions and wall thickening.	58,59
HSCs	Improved left ventricular systolic function and enhanced remodeling.	61,62
MSCs	Prevent deleterious remodeling and initiates speedy recovery.	30,31
iPSCs	Improvement in cardiac function and inhibition of cardiac remodeling.	40,61
CSCs	Reconstitution of a functional ventricular wall, reduced infarct size and LV remodeling halted.	23,55

REFERENCES

[1] Cardiovascular diseases (CVDs) WHO Fact sheet N°317. http://www.who.int/mediacentre/factsheets/fs317/en/index.html, September, **(2011)**.

[2] N.Christoforou, John D.Gearhart; Stem cells and their potential in cell-based cardiac therapies, Prog.Cardiovasc Dis., **49(6)**, 396-413, May-Jun, **(2007)**.

[3] X.H.Parsons, Yang D.Teng, James F.Parsons, Evan Y.Snyder, David B.Smotrich, Dennis A.Moore; Efficient derivation of human cardiac precursors and cardiomyocytes from pluripotent human embryonic stem cells with small molecule induction, J.Vis.Exp., **57**, Nov 3, **(2011)**.

[4] Zhu Wei-Zhong, Kip Hauch, Chunhui Xu, Michael A.Laflamme; Human embryonic stem cells and cardiac repair, Transplant Rev.(Orlando), **23(1)**, 53–68, January, **(2009)**.

[5] C.Alperin, Peter W.Zandstra, Kimberly A.Woodhouse; Engineering cardiac healing using embryonic stem cell-derived cardiac cell seeded constructs, Front Biosci., **12**, 3694-712, May 1, **(2007)**.

[6] Maria Mirotsou, Tilanthi M.Jayawardena, Jeffrey Schmeckpeper, Massimiliano Gnecchi, Victor J.Dzaul; Paracrine mechanisms of stem cell reparative and regenerative actions in the heart, Mol.Cell Cardiol., **50(2)**, 280–289, February, **(2011)**.

[7] F.Zhang Feixiong, Pasumarthi, B.S.Kishore; Embryonic stem cell transplantation: Promise and progress in the treatment of heart disease, BioDrugs., **22(6)**, 361-74 **(2008)**.

[8] I.Kehat Dorit Kenyagin-Karsenti, Mirit Snir, Hana Segev, Michal Amit, Amira Gepstein, et al.; Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes, J.Clin.Invest., **108(3)**, 407-14, Aug, **(2001)**.

[9] J.Han, Kuldip Sidhu; Embryonic stem cell extracts: Use in differentiation and reprogramming, Regen Med., **6(2)**, 215-27, Mar, **(2011)**.

[10] Emanuela Binello, Isabelle M.Germano; Stem cells as therapeutic vehicles for the treatment of high-grade gliomas, Neuro Oncol., **14(3)**, 256-65, Mar, **(2012)**.

[11] C.Y.Fong, Kalamegam Gauthaman, Ariff Bongso; Teratomas from pluripotent stem cells: A clinical hurdle, J.Cell Biochem., **111(4)**, 769-81, Nov1, **(2010)**.

[12] B.Blum, Nissim Benvenisty; The tumorigenicity of human embryonic stem cells, Adv.Cancer Res., **100**, 133-58 **(2008)**.

[13] Z.J.Kastenberg, J.S.Odorico; Alternative sources of pluripotency: Science, ethics and stem cells, Transplant Rev.(Orlando), **22(3)**, 215-22, Jul, **(2008)**.

[14] Leeper J.Nicholas, L.Arwen, Hunter, John P.Cooke; Stem cell therapy for vascular regeneration: Adult, embryonic and induced pluripotent stem cells, Circulation, **122(5)**, 517-526, Aug 3, **(2010)**.

[15] D.K.Singla, A.Ahmed, R.Singla, Yan B.Embryonic; Stem cells improve cardiac function in doxorubicin-induced cardiomyopathy mediated through multiple mechanisms, Cell Transplant., [Epub ahead of print] Mar 22, **(2012)**.

[16] J.Dawson, O.Schussler, A.Al-Madhoun, C.Menard, M.Ruel, I.S.Skerjanc; Collagen scaffolds with or without the addition of RGD peptides support cardiomyogenesis after aggregation of mouse embryonic stem cells, In Vitro Cell Dev.Biol Anim., **47(9)**, 653-64, Oct, **(2011)**.

[17] C.Glass, D.K.Singla; MicroRNA-1 transfected embryonic stem cells enhance cardiac myocyte differentiation and inhibit apoptosis by modulating the PTEN/Akt pathway in the infarcted heart, Am.J.Physiol.Heart Circ.Physiol., **301(5)**, H2038-49, Nov, **(2011)**.

[18] S.Sartore, M.Lenzi, A.Angelini, A.Chiavegato, L.Gasparotto, P.De Coppi, et al.; Amniotic mesenchymal cells autotransplanted in a porcine model of cardiac ischemia do not differentiate to cardiogenic phenotypes; Eur.J.Cardiothorac Surg., **28(5)**, 677-84, Nov, **(2005)**.

[19] F.Tögel, Christof Westenfelder; Adult bone marrow-derived stem cells for organ regeneration and repair, Dev.Dyn., **236(12)**, 3321-31, Dec, **(2007)**.

[20] Il-Hoan Oh, R.Keith Humphries; Concise review: Multidimensional regulation of the hematopoietic stem cell state, Stem Cells, **30(1)**, 82-8, Jan, **(2012)**.

[21] Ketil Lunde, Svein Solheim, Svend Aakhus, Harald Arnesen, Michael Abdelnoor, Torstein Egeland, et al.; Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction, N.Engl.J.ed., **355(12)**, 1199-209, Sep 21, **(2006)**.

[22] Buddhadeb Dawn, Adam B.Stein, Konrad Urbanek, Marcello Rota, Brian Whang, Raffaella Rastaldo, et al.; Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function *PNAS*, **102(10)**, 3771 **(2005)**.

[23] I.Burba, G.I.Colombo, L.I.Staszewsky, M.De Simone, P.Devanna, et al; Histone deacetylase inhibition enhances self renewal and cardioprotection by human cord blood-derived CD34 cells, *PLoS One*, **6(7)**, Jul 18, **(2011)**.

[24] M.G.Engelmann, H.D.Theiss, C.Hennig-Theiss, A.Huber, B.J.Wintersperger, et al; Autologous bone marrow stem cell mobilization induced by granulocyte colony-stimulating factor after subacute ST-segment elevation myocardial infarction undergoing late revascularization: final results from the G-CSF-STEMI (Granulocyte Colony-Stimulating Factor ST-Segment Elevation Myocardial Infarction) trial, J.Am.Coll.Cardiol., **48(8)**, 1712-21, Oct 17 **(2006)**.

[25] Q.Zhao, C.Sun, X.Xu, J.Zhou, Y.Wu, Y.Tian, Z.Yuan, Z.Liu, CD34+ cell mobilization and upregulation of myocardial cytokines in a rabbit model of myocardial ischemia, Int.J.Cardiol., **152(1)**, 18-23, Oct 6, **(2011)**.

Review

[26] M.T.Valarmathi, R.L.Goodwin, J.W.Fuseler, J.M. Davis, M.J.Yost, J.D.Potts; A 3-D cardiac muscle construct for exploring adult marrow stem cell based myocardial regeneration, Biomaterials, **31(12)**, 3185-200, Apr, **(2010)**.

[27] E.Samper, Diez-Juan, J.A.Montero, P.Sepúlveda; cardiac cell therapy: Boosting mesenchymal stem cells effects, Stem cell rev.[epub ahead of print], feb 16, **(2012)**.

[28] A.Charles Goldthwaite; Mending a broken heart: Stem cells and cardiac repair, stem cell information, NIH, **6**, **(2006)**.

[29] G.Vassalli, Tiziano Moccetti; Cardiac repair with allogeneic mesenchymal stem cells after myocardial infarction, Swiss Med.Wkly., 141, May 23, **(2011)**.

[30] Mark F.Pittenger, Bradley J.Martin; Mesenchymal stem cells and their potential as cardiac therapeutics, Circ Res., **95(1)**, 9-20, Jul 9, **(2004)**.

[31] F.B.Zhang, H.T.Yang; Plasticity of bone marrow mesenchymal stem cells differentiating into cardiomyocytes and the potential of cardiac therapeutics, Progress in Physiology, **37(3)**, 199-204, Jul, **(2006)**.

[32] Xi-Ping Huang, Zhuo Sun, Yasuo Miyagi, Heather McDonald Kinkaid, Li Zhang, Richard D.Weisel, et al.; Differentiation of allogeneic mesenchymal stem cells induces immunogenicity and limits their long-term benefits for myocardial repair, Circulation, **122(23)**, 2419-29, Dec 7, **(2010)**.

[33] Dhingra Sanjiv, Huang Xi-Ping, Li Ren-Ke, Challenges in allogeneic mesenchymal stem cell-mediated cardiac repair, Trends Cardiovasc Med., **20(8)**, 263-8, Nov, **(2010)**.

[34] A.J.Nauta, Geert Westerhuis, B.Alwine Kruisselbrink, G.A.Ellie Lurvink, Roel Willemze, Willem E.Fibbe; Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting, Blood, **108(6)**, 2114-20, Sep 15, **(2006)**.

[35] M.S.Choudhery, M.Khan, R.Mahmood, S.Mohsin, S.Akhtar, F.Ali, S.N.Khan, S.Riazuddin; Mesenchymal stem cells conditioned with glucose depletion augments their ability to repair-infarcted myocardium, J.Cell Mol.Med., 1582-4934, Mar 21, **(2012)**.

[36] H.Xu, G.Zhu, Y.Tian, Protective effects of trimetazidine on bone marrow mesenchymal stem cells viability in an ex vivo model of hypoxia and in vivo model of locally myocardial ischemia, J.Huazhong.Univ.Sci.Technolog.Med.Sci., **32(1)**, 36-4, Feb, **(2012)**.

[37] Michael A.Laflamme, Charles E.Murry; Heart Rgeneration, Nature International weekly Journal of Science, **18(473)**, 326-335, May, **(2011)**.

[38] H.Y.Qian, Y.J.Yang, J.Huang, R.L.Gao, K.F.Dou, G.S.Yang, J.J.Li, R.Shen, Z.X.He, M.J.Lu, S.H. Zhao; Effects of tongxinluo-facilitated cellular cardiomyoplasty with autologous bone marrow-mesenchymal stem cells on postinfarct swine hearts, Chin.Med.J.(Engl.), **120(16)**, 1416-25, Aug 20, **(2007)**.

[39] B.Ramesh, D.K.Bishi, S.Rallapalli, S.Arumugam, K.M.Cherian, S.Guhathakurta; Ischemic cardiac tissue conditioned media induced differentiation of human mesenchymal stem cells into early stage cardiomyocytes, Cytotechnology, [Epub ahead of print], Mar 7, **(2012)**.

[40] Yoshinori Yoshida, Shinya Yamanaka; iPS cells: A source of cardiac regeneration, J.Mol.Cell.Cardiol., **50(2)**, 327-32, Feb, **(2011)**.

[41] A.Kunisato, Mariko Wakatsuki, Yuuki Kodama, Haruna Shinba, Isao Ishida, Kenji Nagao; Generation of induced pluripotent stem cells by efficient reprogramming of adult bone marrow cells, Stem Cells Dev., **19(2)**, 229-38, Feb, **(2010)**.

[42] G.T.Huang; Induced pluripotent stem cells-a new foundation in medicine, J.Exp.Clin.Med., **2(5)**, 202-217, Oct 22, **(2010)**.

[43] T.Egashira, Shinsuke Yuasa, Keiichi Fukuda; Induced pluripotent stem cells in cardiovascular medicine, Stem Cells Int., Oct 2, **(2011)**.

[44] J.Zhang, Gisela F.Wilson, Andrew G.Soerens, Chad H.Koonce, Yu.Junying, Sean P.Palecek, James A.Thomson, et al.; Functional cardiomyocytes derived from human induced pluripotent stem cells, Circ Res., **104(4)**, 30-41, Feb 27, **(2009)**.

[45] M.Stadtfeld, et al.; Induced pluripotent stem cells generated without viral integration, Science, **322**, 945–949 **(2008)**.

[46] K.Okita, Masaki Nagaya, Jochen Utikal1, Gordon Weir, Konrad Hochedlinger; Generation of mouse induced pluripotent stem cells without viral vectors, Science, **322**, 949–953 **(2008)**.

[47] B.Dai, W.Huang, M.Xu, R.W.Millard, M.H.Gao, H.K.Hammond, D.R.Menick, M.Ashraf, Y.Wang; Reduced collagen deposition in infarcted myocardium facilitates induced pluripotent stem cell engraftment and angiomyogenesis for improvement of left ventricular function, J.Am.Coll.Cardiol., **58(20)**, 2118-27, Nov 8, **(2011)**.

[48] A.Martinez-Fernandez, T.J.Nelson, Y.Ikeda, A. Terzic; c-MYC independent nuclear reprogramming

favors cardiogenic potential of induced pluripotent stem cells, J.Cardiovasc.Transl.Res., **3(1)**, 13-23, Feb, **(2010)**.

[49] C.A.Sommer, A.G.Sommer, T.A.Longmire, C.Christodoulou, D.D.Thomas, M.Gostissa, F.W. Alt, G.J.Murphy, D.N.Kotton, G. Mostoslavsky; Excision of reprogramming transgenes improves the differentiation potential of iPS cells generated with a single excisable vector, Stem Cells, **28(1)**, 64-74, Jan, **(2010)**.

[50] A.Kuzmenkin, H.Liang, G.Xu, K.Pfannkuche, H. Eichhorn, A.Fatima, et al.; Functional characterization of cardiomyocytes derived from murine induced pluripotent stem cells in vitro, FASEB J., **23(12)**, 4168-80, Dec, **(2009)**.

[51] S.Rupp, Jürgen Bauer, Susanne von Gerlach, Stephan Fichtlscherer, Andreas M. Zeiher, Stefanie Dimmeler, et al; Pressure overload leads to an increase of cardiac resident stem cells, Basic Res. Cardiol., **107(2)**, 252, Mar, **(2012)**.

[52] Torella D.Georgina, M.Ellison, Simón Méndez-Ferrer, Borja Ibanez, Bernardo Nadal-Ginar; Resident human cardiac stem cells: Role in cardiac cellular homeostasis and potential for myocardial regeneration, Nat.Clin.Pract.Cardiovasc.Med., **3(1)**, 8-13, Mar, **(2006)**.

[53] Toru Hosoda; C-kit-positive cardiac stem cells and myocardial regeneration, Am.J.Cardiovasc.Dis., **2(1)**, 58–67, **(2012)**.

[54] A.P.Beltrami, L.Barlucchi, D.Torella, M.Baker, F.Limana, S.Chimenti, et al.; Adult cardiac stem cells are multipotent and support myocardial regeneration, Cell, **114(6)**, 763-76, Sep 19, **(2003)**.

[55] B.Nadal-Ginard, P.Anversa, J.Kajstura, A.Leri; Cardiac stem cells and myocardial regeneration. Novartis Found Symp, **265**, 142-54, **(2005)**.

[56] K.Urbanek, Daniele Torella, Farooq Sheikh, Antonella De Angelis, Daria Nurzynska, Furio Silvestri, et al; Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure, Proc.Natl.Acad.Sci., U.S.A., **102(24)**, 8692-7, Jun 14, **(2005)**.

[57] D.Angert, R.M.Berretta, H.Kubo, H.Zhang, X.Chen, W.Wang, B.Ogorek, M.Barbe, S.R.Houser; Repair of the injured adult heart involves new myocytes potentially derived from resident cardiac stem cells, Circ Res., **108(10)**, 1226-37, May 13, **(2011)**.

[58] L.Barile, E.Messina, A.Giacomello, E.Marbán; Endogenous cardiac stem cells, prog Cardiovasc Dis., **50(1)**, 31-48, Jul-Aug, **(2007)**.

[59] M.A.Laflamme, Kent Y.Chen, Anna V.Naumova, Veronica Muskheli1, James A.Fugate, Sarah K.Dupras, et al.; Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts, Nat.Biotechnol, **25**, 1015–1024 **(2007)**.

[60] Y.Shiba, Kip D.Hauch, Michael A.Laflamme, Cardiac applications for human pluripotent stem cells, Curr.Pharm.Des., **15(24)**, 2791-806 **(2009)**.

[61] R.Bhatia, M.Joshua, M.D.Hare; Mesenchymal stem cells: Future source for reparative medicine, Congest Heart Fail, **11(2)**, 87-91, Mar-Apr, **(2005)**.

[62] S.Arnous, Svein Solheim, Svend Aakhus, Harald Arnesen, Michael Abdelnoor, Torstein Egeland, et al.; Bone marrow mononuclear cells and acute myocardial infarction, Stem Cell Res.Ther., **3(1)**, Jan 17, **(2012)**.

[63] D.W.Losordo, Timothy D.Henry, Charles Davidson, Joon Sup Lee, Marco A.Costa, Theodore Bass et al.; Intramyocardial, autologous CD34+ cell therapy for refractory angina, Circ.Res., **109(4)**, 428-36, Aug 5, **(2011)**.