Journal of Stem Cells & Regenerative Medicine
An exclusive & FREE online journal
www.pubstemcell.com JSRM/Vol6 No.1, 2010; p15-25

Original Article

# Lin-c-kit$^+$ BM-derived stem cells repair Infarcted Heart

Khan M, Mohsin S, Khan SN, Riazuddin S*

National Center of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan

* Sheikh Riazuddin, PhD, National Center of Excellence in Molecular Biology, 87-West Canal Bank Road, University of the Punjab, Lahore, Pakistan. Email: riaz@lhr.comsats.pk, camb1@wol.net.pk

Published online on 05 Apr 2010

## Abstract

Myocardial infarction is one of the leading causes of death worldwide. Bone marrow contains different types of stem cells capable of differentiating into cardiomyocytes. Therefore, we hypothesized that bone marrow contains a lin-c-kit+ progenitor cell pool with the ability to home to the infarcted region and improve the cardiac function. BM-derived stem cells (BMSCs) from Green fluorescent protein (GFP) expressing transgenic mice C57BL/6 (n = 6) were isolated by c-kit labeled Microbeads. Lin-c-kit+ BMSCs co-cultured with rat neonatal cardiomyocytes were able to express cardiac marker and form intercellular connections with rat myocytes. Myocardial infarction was produced in the experimental animals C57BL/6 Wild type (n = 10) by permanent ligation of the Left anterior descending (LAD) artery. BMSCs enriched for c-kit were transplanted in the border zone area of the infarction. Cardiac function analyzed by Millar's Apparatus after 4 weeks of transplantation showed improvement. BMSCs were able to form intercellular connections and new blood vessel formation as determined by Connexin 43 and CD31 respectively. Lin-c-kit$^+$ BMSCs were able to align with the host myocardium, participate in angiogenesis and thus improve the cardiac function.

**Key words**; Stem cells, myocardial infarction, angiogenesis, cardiac function, BMSC differentiation

JSRM www.pubstemcell.com

Copyright © Journal of Stem cells and Regenerative medicine. All rights reserved
JSRM/ 006010300004/Apr 05, 2010.

## 1. Introduction:

Heart is a terminally differentiated organ according to the accepted but never proven paradigm. Heart tissue once lost cannot regenerate accumulating severely hypertrophied myocytes incapable of

supporting the normal cardiac function[1]. Congestive heart failure due to m yocardial infarction is one of the leading causes of death worldwide. In recent years, restoration of cardiac function by Stem cell transplantation has emerged as one of the treatment options. This is supported by the fact that stem cells can home to the damage areas after injury and undergo differentiation[2, 3]. Stem cells are thought to possess unlimited ability of regeneration and can differentiate into any specialized cell type including cardiomyocytes. Stem cells reside in different tissues of the body and possess multilineage potential to form various organ specific cells.

One of the most studied organs in the body for the identification and isolation of stem cells is the bone marrow (BM). BM is an extremely complex organ with several different types of primitive stem and progenitor cells capable of repopulating many nonhematopoietic tissues[4-7]. Thus the BM serves as a central reserve of stem cells in the body and helps to replenish various adult tissues. Different stem cell populations reside within the BM with characteristic morphology and function. Hematopoietic stem cells (HSCs) are one of the best characterized cell types in the BM8 and are considered to be positive for stem cell markers c-kit and CD34. Jackson et al.[9] demonstrated the ability of a fraction of cells highly enriched for HSCs engrafted in the injured heart and expressed cardiomyocytic markers. Similar study was conducted in which genetic techniques were used to follow the fate of hematopoietic stem cells after their transplantation into normal and injured hearts[10]. Mesenchymal stem cells (MSCs) represent another cell type present in the bone marrow with multilineage potential[11]. These cells have been shown to regenerate the heart muscle by differentiating into cardiomyocytes[12-15].

In the present study, we have proposed that a fraction of the BM-derived stem cells separated on the basis stem cell marker c-kit can engraft in the damaged myocardium and improve the overall cardiac function. The study aims to deliver lin-c-kit$^{+}$ HSCs and other stem cell types in the bone marrow at the site of injury highlighting the effectiveness of this approach.

## 2. Materials and Methods

### *2.1. Animals*

All animals were treated according to the *Guidelines for the Care and Use of Laboratory Animals* prepared by the National Center of Excellence in Molecular Biology. The animals were kept in the animal house and all the procedures were approved by the Institutional Review Board (IRB).

### *2.2. Cell Isolation and Characterization*

BM-derived stem cells were isolated from the tibias and femurs of C57BL/6 mice 2-3 months old transgenic green fluorescent protein (GFP) expressing as described in oue previous paper[16]. Briefly, cells were flushed and then incubated with c-kit labeled microbeads and separated by the magnetic activated cell sorting (MACS) procedure. Cells were cultured in Iscove's modified Dulbecco's medium (IMDM) supplemented with 20% fetal bovine serum and antibiotics at 37° C in humid air and 5% CO2.

### *2.3. Flow Cytometry*

For flow cytometry analysis, BMSCs were washed with PBS (pH = 7.4) and incubated in the dark for 30 min at room temperature with c-kit or CD117-PE and lin PerCP antibodies. The specific fluorescence of 10,000 cells was analyzed on FACScalibur (Becton Dickinson, USA) using Cell Quest Pro software.

Copyright © Journal of Stem cells and Regenerative medicine. All rights reserved
JSRM/ 006010300004/Apr 05, 2010.

*2.4. Co-culture with Neonatal Cardiomyocytes*

Neonatal rat cardiomyocytes were isolated from *Sprague Dawley* rat pups (1-2 days old, n = 15 per isolation). The cells were separated with neonatal cardiomyocyte isolation system (Worthington) as described by Xu et al[17]. Lin-c-kit+ BMSCs were co-cultured with neonatal cardiomyocytes in a ratio of 1:40. Cells were grown for a week followed by immunocytochemical analysis.

*2.5. Immunocytochemistry*

Immunocytochemistry was done as described by Kudo et al[18] after passage 1 (day 4). Primary antibodies used were rabbit polyclonal anti-c-kit (Chemicon) and goat polyclonal anti-CD34 ( Santa Cruz) antibodies. Evidence for cardiac differentiation of BMSCs was assessed by staining with goat polyclonal anti-connexin-43 (Santa Cruz) and α-sarcomeric actin (Sigma) antibodies. Secondary antibodies of donkey anti-rabbit IgG, anti-goat IgG and anti-mouse IgG (Jackson Immunoresearch) conjugated with rhodamine were applied. Nuclei were stained with 4`, 6-diamidino-2-phenylindole (DAPI). Fluorescent images were obtained with an Olympus BX-61 microscope equipped with DP-70 digital camera (Olympus).

*2.6. Myocardial infarction model and Cell transplantation*

Female C57BL/6 mice 2-3 months old were divided into three groups. Group I represented normal mice (n = 10), Group II as control mice (n = 10) and Group III mice (n = 10) as the cell transplantation group. Myocardial infarction was produced in Group II and III animals according to the procedure described by Lanza et al[19]. Briefly, the mice were anesthetized with intraperitoneal injection of sodium pentobarbital (40mg/kg). Body temperature was carefully monitored and the heart was exposed. Left anterior descending (LAD) artery was ligated with 6-0 silk suture 1mm from the tip of the normally positioned left auricle. Lin-c-kit+ BMSCs were transplanted in the border area of the infarct zone. The cell concentration used was 1 x 106 cells/ ml. Control mice underwent sham operations with saline injections.

*2.7. Gene analysis of the transplanted hearts*

RNA was extracted from all the experimental groups using trizole reagent (Invitrogen Corporation) and quantified using ND-1000 spectrophotometer (NanoDrop Technologies). cDNA synthesis was carried out from 1µg of RNA sample using M-MLV reverse transcriptase (Invitrogen Corporation). RT-PCR analysis for MEF-2 and GATA-4 was carried out using a GeneAmp PCR system 9700 (Applied Biosystem) with GAPDH as internal control. The sequences (5'-3') for the primer pairs and their product lengths (bp) have been mentioned in table 2.

*2. 8. Assessment of heart function*

Heart function was analyzed after 4 weeks of stem cell administration. Mice were anesthetized with sodium pentobarbital (40mg/kg) by intraperitoneal injection. The right carotid artery was cannulated with a micro tip pressure transducer catheter (SPR-839, Millar Instrument) connected to MPCU-400 P-V signal conditioning hardware for data acquisition. The inferior vena cava (IVC) was exposed and IVC occlusion (IVCO) was performed by external compression. Hemodynamic parameters and PV loops were recorded during steady state. LV systolic function was evaluated by Pmax and dP/dtmax. LV diastolic function was evaluated by end-diastolic pressure. Hemodynamic parameter analysis was carried out using Millar's PVAN software (Version 3.3).

*2. 9. Measurement of Infarct size*

Fixed hearts were embedded in paraffin and sections were cut from the mid-Left Ventricle (LV) and base. All sections were stained with Masson's Trichrome. Images of the LV area of each slide were taken by an Olympus BX-61 microscope equipped with Digital Camera DP-70 (Olympus). Fibrosis and total LV area of each image were measured using the Image

JSRM
www.pubstemcell.com

Copyright © Journal of Stem cells and Regenerative medicine. All rights reserved
JSRM/ 006010300004/Apr 05, 2010.

Pro Plus and the percentage of the fibrotic area was calculated as (fibrosis area/ LVarea) x 100.

**TABLE 1: Hemodynamic Parameters**

| GROUPS | HEART RATE (BPM) +/- SEM | | END SYSTOLIC VOLUME (RVU) +/- SEM | | END DIASTOLIC VOLUME (RVU) +/- SEM | | MAX PRESSURE (mmHg) +/- SEM | | END DIASTOLIC PRESSURE (mmHg) +/- SEM | | dP/dT (mmHg/sec) +/- SEM | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 Wks | 8 Wks | 4 Wks | 8 Wks | 4 Wks | 8 Wks | 4 Wks | 8 Wks | 4 Wks | 8 Wks | 4 Wks | 8 Wks |
| **GROUP I (Normal)** | 515 +/- 7 | 521 +/- 11 | 12.26 +/- 0.27 | 12.82 +/- 1.32 | 13.59 +/- 0.42 | 12.11 +/- 1.23 | 98 +/- 7.56 | 105 +/- 6.51 | 4.3 +/- 0.19 | 5.1 +/- 2.12 | 7463 +/- 394 | 7781 +/- 401 |
| **GROUP II (Control)** | 343 +/- 16 | 325 +/- 22 | 20.52 +/- 0.01 | 18.92 +/- 1.44 | 19.48 +/- 0.04 | 18.01 +/- 1.11 | 71.01 +/- 5.32 | 73.44 +/- 8.71 | 12.61 +/- 0.45 | 11.66 +/- 1.21 | 4237 +/- 446 | 4589 +/- 231 |
| **GROUP III (Treated)** | 465 +/- 12* | 471 +/- 20* | 15.83 +/- 0.06* | 13.43 +/- 0.33* | 17.32 +/- 0.02* | 15.41 +/- 0.74 | 85.01 +/- 5.21* | 89.92 +/- 4.12 | 7.21 +/- 0.23* | 5.52 +/- 0.91* | 6778 +/- 396.44 * | 7210 +/- 310.21 * |

**TABLE 2: Primer Sequences**

| No. | Genes | Product Size (bp) | Primer Sequence (5'-3') |
|---|---|---|---|
| 1 | GAPDH | 370 | CTCTTGCTCTCAGTATCCTTG |
| | | | GCTCACTGGCATGGCCTTCCG |
| 2 | MEF-2 | 178 | CACGCCTGTCACCTAACATCC |
| | | | TGTTAGCTCTCAAACGCCACAC |
| 3 | GATA-4 | 131 | ATGGGACGGGACACTACCTG |
| | | | GCAGTTGGCACAGGAGAGG |

*2. 10. Immunohistochemistry*

Hearts were removed from the experimental animals after 1 and 4 weeks of stem cell transplantation. Hearts were fixed in 4% paraformaldehyde and then snap frozen in liquid nitrogen. Sections were cut at 5-8µm in a Cryostat (MICROME, Germany, Gmbh) at -20°C. Heart sections were stained with rabbit polyclonal anti-c-kit (Santa Cruz), goat polyclonal anti-CD34 (Santa Cruz), goat polyclonal anti-connexin-43 (Santa Cruz) and mouse anti-CD31 (Chemicon) antibodies. Cardiac muscle was stained with α-sarcomeric actin (Sigma) antibody. Secondary antibodies of donkey anti-rabbit IgG, anti-goat IgG and anti-mouse IgG (Jackson Immunoresearch) conjugated with rhodamine and FITC were applied.

JSRM www.pubstemcell.com

Copyright © Journal of Stem cells and Regenerative medicine. All rights reserved
JSRM/ 006010300004/Apr 05, 2010.

*2.11. Statistical Analysis*

Quantitative data was obtained from 2 coverslips each from 3 separate experiments for BMSC characterization. Five random fields per coverslip were analyzed and the data was expressed as mean ± SEM. Hemodynamic parameters were assessed by one-way analysis of variance ANOVA (*p* value of less than 0.05 was considered statistically significant). Analysis of percentage of fibrosis and comparison of vascular density between groups was performed by student's unpaired t-test (*p* value of less than 0.05 was considered statistically significant).

## 3. Results

*3.1. Characterization of lin-c-kit+ BMSCs*
BM-derived stem cells were isolated with c-kit labeled microbeads from C57BL/6 GFP expressing transgenic mice. Cells in culture demonstrated a heterogeneous population that consisted of small, rounded and spindle shaped cells. The expression of c-kit and CD34 in BMSCs was 69% ± 5 and 40.6% ± 6.4 respectively (Figure 1 A and B).The isolated BM stem cells were gated on the basis of size, granularity and surface marker expression. The gated cells were found to be lineage negative (Lin-) and positive for CD-117 or c-kit (Figure 1 C and D).

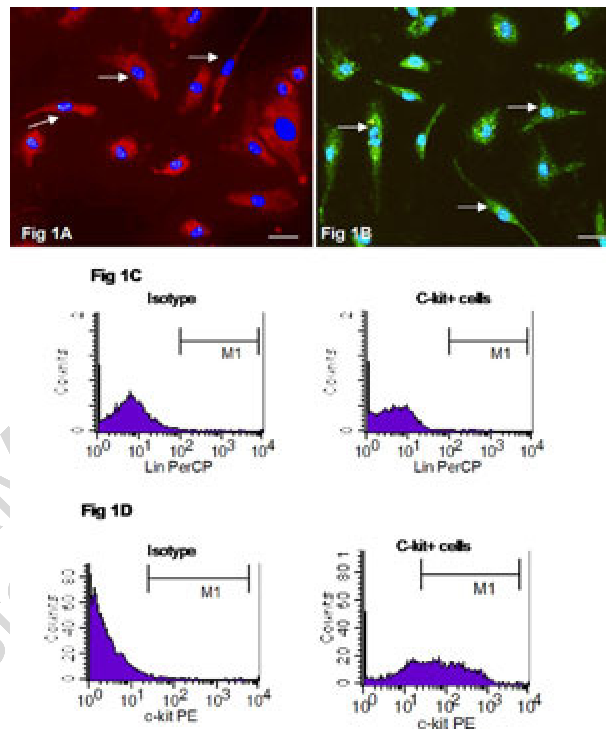
*3.2. In vitro expression of Connexin-43 and sarcomeric actin*

BMSCs positive for c-kit were co-cultured with neonatal rat cardiomyocytes. The BMSC-cardiomyocyte co-culture system was stained with connexin-43 to observe the ability of the cells to form intercellular connections with cardiomyocytes (Figure 2 A and B). The co-culture system was also stained with α-sarcomeric actin confirming the expression of cardiac marker in BMSCs (Figure 2 C and D).

*3.3. Fate of lin-c-kit+ BMSCs after transplantation*

The presence of GFP positive cells in the infarcted area 1 week after transplantation confirmed that the lin-c-kit+ BMSCs were able to move to the ischemic myocardium. The observed cells were found to be positive for c-kit and CD34 confirming the fate of the transplanted cells (Figure 3 A-C). Gene analysis revealed and increase in the expression of MEF-2 and GATA -4

**Figure 1**




**Characterization of BM-derived Lin-c-kit$^+$ .** A) Cells positive for c-kit (arrows). B) Cells positive for CD34 (arrows). Nuclei stained with DAPI. (400x, Scale bar ~ 20μm). C and D) Flow cytometric histograms showing the immunophenotype of BM-derived stem cells. Cells were negative for lin PerCP (0.25%) and positive for CD117 PE (60.5%). Isotype controls show non-specific fluorescence recorded lower than 102 region and for this reason fluorescence above 102 was read as specific.

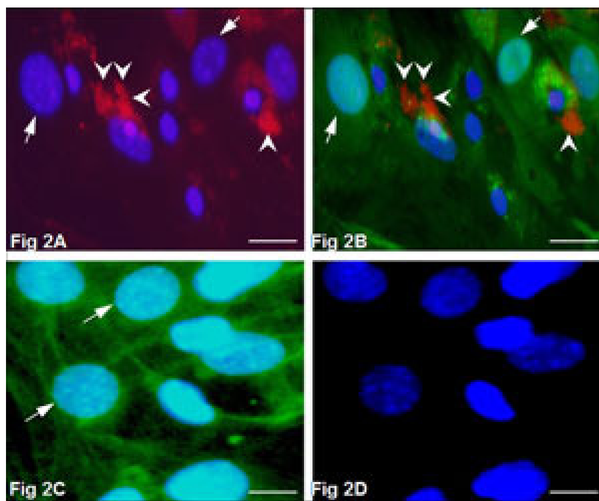
*3.4. Hemodynamic Parameters*

Cardiac function of the mice transplanted with lin-c-kit+ cells were assessed after 4 weeks with Millar's apparatus. Different hemodynamic parameters\including heart rate, endsystolic volume Ves, end-diastolic volume Ved, end-diastolic pressure, maximum pressure (Pmax) and dp/dtmax is given in Table 1. Heart function in the stem cell treated group (Gp III) was significantly improved as

Copyright © Journal of Stem cells and Regenerative medicine. All rights reserved
JSRM/ 006010300004/Apr 05, 2010.

compared with the sham operated control hearts (Gp II). Ves and Ved of group II were shifted rightward and showed a shallow end systolic pressure volume relationship (ESPVR), thus indicating a character of dilated cardiomyopathy. Left ventricle systolic function as determined by Pmax, dp/dtmax increased significantly in group III as compared to the group II. All the hemodynamic parameters of GP III showed improvement indicating a better performance of the treated heart. There was an improved left ventricular function with increased wall mobility and thickness.

**Figure 2**

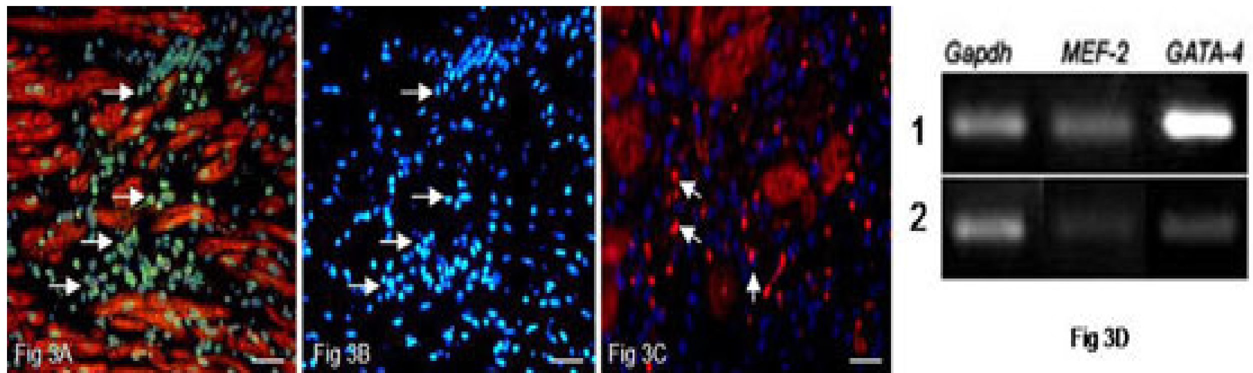



www.pubstemcell.com

**Lin-c-kit⁺ cells form intercellular connection with cardiomyocytes.** A) Lin-c-kit⁺ with multiple nucleoli (arrow), Expression of connexin-43 in between Lin-c-kit⁺ cells and cardiomyocytes (arrowheads). B) Merge in tri band filter. C) Transdifferentiated Lin-c-kit⁺ cells expressing α-sarcomeric actin (arrows). D) Nuclei stained with DAPI (400x, Scale bar ~ 20µm)

Copyright © Journal of Stem cells and Regenerative medicine. All rights reserved
JSRM/ 006010300004/Apr 05, 2010.

**Figure 3**




**Fate of the transplanted Lin-c-kit$^+$ stem cells.** A) c-kit positive cells within the infarcted region, myocytes with sarcomeric actin. B) Nuclei stained with DAPI. C) CD34 positive cells within infarcted region (arrows). (200x, Scale bar ~ 20µm). D) Gene analysis of the stem cell transplanted hearts (1) show elevated levels of MEF-2 and GATA-4 compared to control hearts (2)

*3.5. Reduction of fibrosis after lin-c-kit+ BMSC transplantation*

The sections were stained with Masson's Trichrome to measure fibrosis in normal, infarcted and treated groups. The normal heart section was negative for fibrosis (Figure 4 A) while the infarcted heart isolated from sham operated animals showed infarction with 24.5% ± 3.5 of fibrotic area as shown in figure 4 B. Four weeks after myocardial infarction and the administration of lin-c-kit+ cells the percentage of fibrosis was significantly reduced to 7.5% ± 2.9 in the stem cell treated group (Figure 4 C).

*3.6. Formation of new muscle, intercellular connections and angiogenesis*

Lin-c-kit+ BMSCs were able to home to the infarcted area and form new muscle in the infarcted region of the heart as demonstrated by the co-localization of GFP and α-sarcomeric actin (Figure 5 A-D). Heart sections were also stained with connexin-43 which is a marker for gap junction proteins. The transplanted cells were positive for connexin-43 indicating that the donor cells not only settled in the infarcted myocardium but also formed connections between the treated myocardium (Figure 6 A-C). Connexin-43 was apparent in the cell cytoplasm and at the surface of closely aligned lin-c-kit+ BMSCs and rat myocytes. Sections obtained from treated animals after 4 weeks of stem cell transplantation showed significant increase in the new blood vessel formation. The capillary density at low-power magnification 200x in infarcted control is 8.6 ± 1.7. CD31+ cells in the stem cell treated group are 39.6 ± 1.4 (Figure 6 D-F).

**Figure 4**

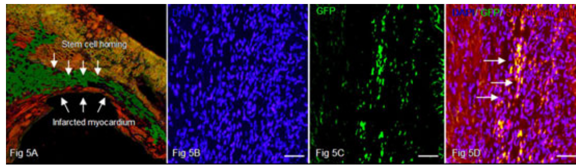
**Masson's trichrome staining of the heart sections after 4 weeks shows infarcted region of the Left**

**ventricle (LV) wall with fibrosis**: A) Normal section of heart (Group I) with no fibrosis. B) Group II heart. C) Group III heart. The $p$ values less than 0.05 were considered significant.

www.JSRM.pubstemcell.com

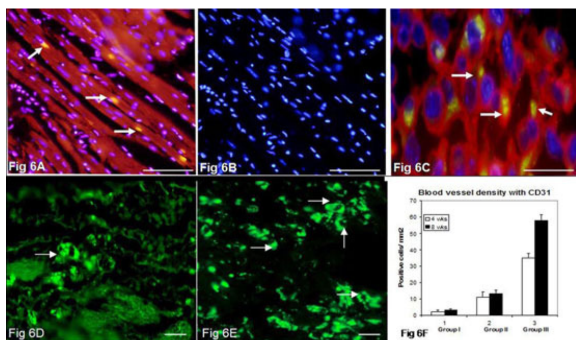
Copyright © Journal of Stem cells and Regenerative medicine. All rights reserved
JSRM/ 006010300004/Apr 05, 2010.

**Figure 5**




**Stem cell homing and myocardial repair.** A) Lin-c-kit$^{+}$ cells expressing GFP home to the infarcted area and form new heart tissue (green). B) Nuclei stained with DAPI. C) GFP+ Lin-c-kit$^{+}$ BM-derived stem cells D) GFP+ Lin-c-kit$^{+}$ BM-derived stem cells appear yellow after co-localization of sarcomeric actin (red). (200x, Scale bar ~ 20µm)

**Figure 6**




**Expression of gap junction protein and angiogenesis.** A) Border zone area. B and C) Regenerating myocardium stained with Connexin 43, sarcomeric actin and DAPI, arrows indicate cell contacts (1000x, Scale bar ~ 20µm). D) Group II Infarcted heart with saline injection. E) Newly formed blood vessels within the infarcted area in Group III heart (arrows). (200x, Scale bar ~ 20µm). F) Blood vessel density analysis for the expression of CD31 after 4 and 8 weeks of stem cell transplantation. *P value of $< 0.05$ vs group II.

## 4.Discussion

In the present study, lineage negative (Lin-) BM cells from transgenic mice expressing enhanced green fluorescent protein were separated by using magnetic bead isolation system on the basis of c-kit expression. The GFP+ cells were able to express c-kit and CD34 and after transplantation in infarcted myocardial tissue improved the cardiac function.

Our decision to separate BM cells on the basis of c-kit was merited by the fact that c-kit receptor is displayed on the cell surface of different stem cells. The c-kit receptor is broadly distributed among the hematopoietic stem cells and is also found in other tissue specific stem cells[20] . Studies in mice have shown that functional SCF and c-kit are critical in the survival and development of stem cells involved in hematopoiesis[21, 22] . Thus the presence of c-kit receptor on BM-derived stem cells indicates that these cells have the ability to mobilize from the bone marrow mediated by SCF as well as other growth factors. A number of reports have suggested that HSCs negative for c-kit receptor possess declined long term repopulating ability[23] .

The isolated cells were also expressed CD34 which is present on different stem and progenitor cells. In humans, approximately 60% to 75% of CD34+ hematopoietic cells co-express the c-kit receptor[24] . This indicates that the CD34+ stem cells are closely associated with the c-kit receptor. Many researchers have demonstrated the potential of bone marrow mononuclear cells or the endothelial progenitor cells, and CD34+ cells for the regeneration of damaged myocardium[25, 26] .

The hemodynamic parameters measured in different experimental groups showed that the hearts treated with stem cells performed better than the sham operated hearts. Analysis of end-systolic volume and end-diastolic volume demonstrated an improvement in the contractility of the infarcted heart after administration of lin-c-kit+ cells. Heart function in sham operated group showed decreased contractility evident by a shift towards right indicating dilated cardiomyopathy. Left ventricular systolic function as analyzed by calculating maximum pressure and maximum dp/dt demonstrated an improvement after treatment with stem cells. The observed augmentation of cardiac function is in accordance with the similar findings demonstrating improved function in

www.pubstemcell.com

Copyright © Journal of Stem cells and Regenerative medicine. All rights reserved
JSRM/ 006010300004/Apr 05, 2010.

the cryodamaged and infarcted heart tissues[18 - 27] .

The present study demonstrated that the transplanted cells expressed angiogenic markers essential for the restoration of cardiac function. Sections from the treated heart showed immunoreactivity for CD31 which is one of the markers for neoangiogenesis. Angiogenesis is a complex physiological process that leads to the formation of a three dimensional capillary network. It has been long recognized that the activation of angiogenesis is associated with a number of distinct phenotypic changes in endothelial cells that enable them to enter an angiogenic cascade leading to cardio protection[28] . The expression of CD31 was significantly increased after lin-c-kit$^+$ cells transplantation, suggesting its role in the repair of damaged vessels that play a vital role in the proper cardiac functioning. Kamihata et al[29] has demonstrated that bone marrow cells can induce angiogenesis by supplying angioblasts and angiogenic factors.

Many studies have shown that the MSCs transplanted into the myocardial environment could express myogenic specific protein such as α-sarcomeric actin, myosin heavy chain, desmin and Troponin T30 . However, in the present study it was demonstrated that transplanted cells were positive for gap junction protein connexin-43. Connexin-43 protein is responsible for intercellular connections and electrical coupling through the generation of plasma-membrane channels between myocytes[31, 32] . Lin-c-kit$^+$ transplanted BMSCs also demonstrated their differentiation into cardiac phenotypes. The positive expression for α-sarcomeric actin and the gap junction protein indicates that the transplanted cells not only settled in the infarcted region but also formed connections between the host myocardium.

In conclusion, selection of BM-derived stem cells on the basis of c-kit demonstrated that the BM has a lin-c-kit$^+$ progenitor cell pool capable of repairing the damaged heart tissue. The current findings have provided compelling evidence that our approach has relevant implications for human disease. The engrafted cells acquired endothelial and myocardial phenotypes and this differentiation led to the improvement of cardiac function in mice.

**Acknowledgments**

We thank our colleagues for the critical review of our manuscript.

**Sponsors/Grants**

This study was supported by the Higher Education commission Islamabad, Pakistan and the Ministry of Science and Technology, Islamabad, Pakistan.

JSRM www.pubstemcell.com

**References**

1.Sussman MA, Anversa P. Myocardial aging and senescence: Where have the stem cells gone? *Ann. Rev. Physiol.* 2004; 66: 29-48.

2.Lutz M, Rosenberg M, Kiessling F, Eckstein V, Heger T, Krebs J, Ho AD, Katus AH, Frey N. Local injection of stem cell factor (SCF) improves myocardial homing of systemically delivered c-kit+ bone marrow-derived stem cells. *Cardiovasc. Res.* 2008; 77(1): 143-150.

3.Penn MS, Zhang M, Deglurkar I, Topol EJ. Role of stem cell homing in myocardial regeneration. *Int. J. Cardiol.* 2004; 95: S23-25.

4.Goodell MA, Jackson KA, Majka SM, Mi T, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Micheal LH, Hirshci KK. Stem cell plasticity in muscle and bone marrow. *J. Clin. Invest.*2002; 109: 1291-1302.

5.Krause DS. Plasticity of marrow-derived stem cells. *Gene Therapy* 2002; 9:754-758.

6.Scintu F, Reali C, Pillai R, Badiali M, Sanaa MA, Argiolu F, Ristaldi MS, Sogos V. Differentiation of human bone marrow stem cells into cells with a neural phenotype: Diverse effects of two specific treatments. *BMC Neurosci.* 2006; 16: 7-14.

7.Tomita Y, Makino S, Hakuno D, Hattan N, Kimura K, Miyoshi S, Murata M, Ieda M, Fukuda K. Application of

Copyright © Journal of Stem cells and Regenerative medicine. All rights reserved
JSRM/ 006010300004/Apr 05, 2010.

mesenchymal stem cell-derived cardiomyocytes as bio-pacemakers: Current status and problems to be solved. *Med. Biol. Eng. Comput.* 2007; 45(2): 209-220.

8.Thoren LA, Liuba K, Bryder D, Nygren JM, Jensen CT, Qian H, Antonchuk J, Jacobsen SE. Kit regulates maintenance of quiescent hematopoietic stem cells. *J. Immunol.* 2008; 180(4): 2045-2053.

9.Jackson, K.A., Majka, S.M., Mi, T., Wang, H., Pocius, J., Hartley, C.J., Majesky, M.W., Entman, M.L., Micheal, L.H., Hirshci, K.K. and Goodell, M.A. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J. Clin. Invest.* 2001; 107: 1395-1402.

10.Wang Y, Haider HK, Ahmad N, Xu M, Ge R, Ashraf M. Combining pharmacological mobilization with intramyocardial delivery of bone marrow cells over-expressing VEGF is more effective for cardiac repair. *J. Mol. Cell Cardiol.* 2006; 40: 736-745.

11.Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craing S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; 284: 143-147.

12.Ball SG, Shuttleworth AC, Kielty CM. Direct cell contact influences bone marrow mesenchymal stem cell fate. *Int. J. Biochem. Cell Biol.* 2004; 36(4): 714-727.

13.Li X, Yu X, Lin Q, Deng C, Shan Z, Yang M, Lin S. Bone marrow mesenchymal stem cells differentiate into functional cardiac phenotypes by cardiac microenvironment. *J. Mol. Cell Cardiol.* 2007; 42(2): 295-303

14.Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation*2002; 105: 93-98.

15.Uemura R, Xu M, Ahmad N, Ashraf M. Bone marrow stem cells prevent left ventricular remodeling of ischemic heart through paracrine signaling. *Circ. Res.* 2006; 98: 1414-1421.

16.Khan M, Manzoor S, Mohsin S, Khan SN, Ahmad FJ. IGF-1 and G-CSF complement each other BMSCs migration towards infarcted myocardium in a novel in vitro model. *Cell Biol. Int.* 2009; 33(6): 650-657.

17.Xu M, Wani M, Dai YS, Wang J, Yan M, Ayub A, Ashraf M. Differentiation of bone marrow stromal cells into the cardiac phenotype requires intercellular communication with myocytes. *Circulation* 2004; 110: 2658-2665.

18.Kudo M, Wang Y, Wani MA, Xu M, Ayub A, Ashraf M. Implantation of bone marrow stem cells reduces the infarction and fibrosis in ischemic mouse heart. *J. Mol. Cell Cardiol.* 2003; 35: 1113-1119.

19.Lanza R, Moore MA, Wakayama T, Perry AC, Shieh JH, Hendrikx J, Leri A, Chimenti S, Monsen A, Nurzynska D, West MD, Kajstura J, Anversa P. Regeneration of the infarcted heart with stem cells derived by nuclear transplantation. *Circ. Res.* 2004; 94: 820-827.

20.Zhang W, Zhang G, Jin H, Hu R. Characteristics of bone marrow-derived endothelial progenitor cells in aged mice. *Biochem. Biophys. Res.Comm.* 2006; 348: 1018-1023.

21.Huang E, Nocka K, Beier DR, Chu TY, Buck J, Lahm HW, Wellner D, Leder P, Besmer P. The hematopoietic growth factor KL is encoded by the *SI* locus and is the ligand of the *c-kit* receptor, the gene product of the *W* locus. *Cell* 1990; 63: 225-233.

22.Linnekin D. Early signaling pathways activated by *c-kit* in hematopoietic cells. *Int. J. Biochem. Cell Biol.* 1999; 31: 1053-1074.

23.Li CL, Johnson GR. Murine hematopoietic stem and progenitor cells: Enrichment and biologic characterization. *Blood* 1995; 85(6): 1472-149.

24.Ashman LK, Cambareri AC, To LB, Levinsky RJ, Juttner CA. Expression of the YB5.B8 antigen (c-kit proto-oncogene product) in normal human bone marrow. *Blood* 1991; 78(1): 30-37.

25.Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Frofang K. Autologous stem cell transplantation in acute myocardial infarction. The ASTAMI randomized controlled trial: Intracoronary transplantation of autologous mononuclear bone marrow cell study, design and safety aspect. *Scand. Cardio. J.* 2005; 39: 150-158.

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

27.Ciulla MM, Ferrero S, Lazarri L, Pacchiana R, Paliotti R, Gianelli U, Busca G, Esposit A, Bosari S, Magrini F, Rebullar P. The translocation of marrow MNCs after experimental myocardial cryoinjury is

JSRM www.pubstemcell.com

Copyright © Journal of Stem cells and Regenerative medicine. All rights reserved
JSRM/ 006010300004/Apr 05, 2010.

proportional to the infarcted area. *Transfusion* 2004; 44:239-244.

28.Ding S, Merkulova-Rainon T, Han ZC, Tobelem G. HGF receptor up-regulation contributes to the angiogenic phenotypes of human endothelial cells and promotes angiogenesis in vitro. *Blood* 2003; 101: 4816-4822.

29.Kamihata H, Matsubara H, Nishiue T. 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* 2001; 104(9): 1046-1052.

30.Tomita S, Mickle DA, Weisel RD. Improved heart function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation. *J. Thorac. cardiovac. sur.* 2002; 123: 1132-1140.

31.Beardsle MA, Laing JG, Beyer EC, Saffitz JE. Rapid turnover of connexin 43 in the adult rat heart. *Circ. Res.* 1998; 83: 629-635.

32.Musil LS, Le AN, VanSlyke JK, Roberts LM. Regulation of connexin degradation as a mechanism to increase gap junction assembly and function. *J. Biol. Chem.* 2000; 275: 25207-25215.

JSRM
www.pubstemcell.com

Copyright © Journal of Stem cells and Regenerative medicine. All rights reserved
JSRM/ 006010300004/Apr 05, 2010.