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Chapter 7

Stem Cell-Based (Auto)Grafting: From Innovative Research Toward Clinical Use in Regenerative Medicine

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Additional information is available at the end of the chapter

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1. Introduction

In brief, adult stem cells (SCs) give rise to repopulation (engraftment) of recipient's bone marrow (BM) followed by complete and long-term reconstitution of hematopoiesis. In addition, totipotent SCs are also capable of colonizing different tissues (homing). Initial studies showed that "implantation" of autologous SCs into damaged and ischemic area induces their homing and subsequent "transdifferentiation" into the cell lineages of host organ, including collateral vessel formation. Angiogenesis growth factors – or genes encoding these proteins – promote the development of collateral micro-angiogenesis or "therapeutic neovascularization" [1– 5].

Generally, SC transplant involves the administration of high-dose chemotherapy (conditioning regimen) and (re)infusion of collected cells in order to obtain an abolition of disease, as well as to get hematopoietic reconstitution and clinical improvement of patient. SC transplant with reduced-intensity conditioning (RIC) can be offered to patients who are ineligible for high-dose conditioning because of their age or comorbidities [2]. Hematological diseases have so far been the most common indication of this treatment modality; it has been less often used for nonmalignant disorders. Nowadays BM and peripheral blood (PB) derived SC transplants are more common in adult allogeneic or autologous setting [2, 6– 8]. Umbilical cord blood (UCB) transplants have provided hopeful results in pediatric setting mainly when a matched unrelated SC donor is not obtainable [9–12].

In clinical practice, SCs can be collected by: (a) multiple aspirations from BM; (b) harvesting PB after mobilization with chemotherapy and/or growth factors (rHuG-CSF), and (c) by specific processing from UCB. SCs collected from the stated sources can be clinically applied

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(transplanted) immediately following harvesting (allogeneic setting) or after a long-term storage in frozen state – cryopreservation (autologous setting) [2].

2. Stem cell transplants – A short chronological consideration

Independent SC-researchers recognized that all blood cells originate from one primitive BM cells (totipotent SC) located in marrow – space where the entire hematopoiesis takes place. Initial animal studies revealed that the BM was the organ most sensitive to the damaging effect of gamma irradiation [2]. Quickly, it became clear that (re)infusion of marrow cells or SCs could rescue lethally irradiated animals. Thomas with colleagues started on, and after that optimized (for this initial period) the BM transplant (BMT) program for humans and published in 1957 the first clinical results [13]. During the late 1950s have been also described the first syngeneic BMT in patients with leukemia [14]. Mathe and coworkers published the treatment of patients by allogeneic SC transplant after of accidental irradiation [15]. These transplants were performed before the discovery of major histocompatibility (MCH) system. In addition, it is not excluded that the observed recovery of some patients were a result of the recovery of autologous hematopoietic system [16]. The first successful BMT (allogeneic) was performed on a child with severe combined immunodeficiency disease (SCID). Cells were collected from his sister, and his immune system was restored following transplant [2]. However, in most cases transplants in humans have been unsuccessful (because of the graft rejection or expansion of the Graft versus Host Disease – GvHD).

The modern era of SC transplants – as a standard therapy – started with fundamental discovery and permanent progress in the knowledge of MHC, that is human leukocyte antigen (HLA) system [17]. These antigens give the body's immune system the ability to determine what belongs and what does not belong to the human body. Whenever the immune system does not recognize antigens expressed on a cell surface, it produces antibodies and other mediators to destroy the cells with non-recognizable antigens. In order for BMT to work, the recipient's immune system must not try to destroy the donated cells. This comprises that the HLA antigens on the donated SCs have to be identical or extremely similar to the antigens of the recipient's cells. Even with this careful HLA matching, transplant may still fail because recipient's immune system destroys transplanted cells (graft rejection) or donor's cells attempt to damage recipient's target cells (GvHD) [2]. Thomas and coworkers almost immediately published positive results of the first allogeneic BMT in patients with hematologic malignancies – using cells from donors selected on the basis of the HLA system [18]. After all, Thomas ED was awarded the Nobel prize in medicine (1990) for his overall pioneering work on BMT topic. He was awarded the prize because of his numerous triumphant activities in both, experimental and clinical transplant setting.

In addition to marrow, PB has gained popularity as a SC source since their initial introduction in the early 1980s [19]. Over the past decades, the use of these transplants has expanded rapidly [6– 8]. Using umbilical cord blood (UCB) derived SCs, successful transplant occurred the first time in the treatment of Fanconi anemia and other disorders later than [9–12]. It is known that

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only about one-third of patients have related HLA-matched donor. For that reason, some sources of allogeneic donors – including unrelated HLA-compatible individuals, have to be considered as the possible alternative. As a result, National Marrow Donor Program's registries of volunteer donors has been created and data accumulated by organizing a unique database for potential donors (Bone Marrow Donors Worldwide – BMDW) [20].

The late 1990s brought a new apprehension regarding the biology and related novel clinical potential of SCs. Researchers began to realize that manipulation of adult animal tissues could sometimes yield previously unsuspected cell types; for example, that some BM derived SCs could be turned into cardiomyocytes, hepatocytes or nerve and other somatic cells – phenomenon known as the SC "plasticity". Finally, using SC plasticity, cell–based therapies for treatment of the ischemic heart diseases started through beginning of the new millennium and currently are in an expansion phase in the other fields of regenerative medicine [21–30].

3. Adult stem cells: New concepts in phenotypes and functionality

To prove that SCs derived from BM and PB, including hematopoietic SCs, are indeed transdifferented or transformed into solid organ specific cells, several conditions must be met:

- The origin of the exogenous cell integrated into solid-organ time must be documented by marking the cell, preferably at the single-cell level;
- Cell should be processed with a minimum of *ex vivo* manipulation which may make them more susceptible to crossing lineages;
- The exogenous cells must be shown to have become an integral morphological part of the newly acquired tissue;
- Transdifferented cells must have shown to acquire the function of the particular organ into which it has been integrated both, by expressing organ-specific proteins and by showing specific organ function.

Nevertheless, taking into consideration their common features described in the literature, it is very likely that various investigators have described overlapping populations of developmentally early SCs that are closely related. Our intention is to make a clear distinction between three different types of adult SCs.

4. The concept of hematopoietic stem cells

Organ/tissue specific niche (like in BM, liver, etc) exists as a deposit (storage) of the adult SCs in a specific location [31]. These cells are circulating in a very low number in the PB. Accumulating evidence suggests that SCs may also actively migrate/circulate in the postnatal period of life. SC trafficking/circulation may be one of the crucial mechanisms that maintains the pool of SCs dispersed in SC-niches of the same tissue, that are spread throughout different ana-

tomical areas of the body. This phenomenon is very well described for hematopoietic SCs (HSCs), but other, already tissue committed SC or TCSC (for example, endothelial, skeletal muscle or neural SCs) are probably circulating as well. BM is the home of migrating SCs with not only HSCs within their niches, but also a small number of TCSC, which might be the reason why many authors think that HSC may transdifferentiate, although we do not have a direct proof for that. They might have plasticity, but not necessarily the "potential for transdifferentiation" [32–39]. What is differentiated in the tissue of injection might be TCSC characteristic for that tissue. It has been shown that number of these cells is decreased with ageing (long living and short living mice and humans). It would be interesting to identify genes that are responsible for tissue distribution/expansion of TCSC. These genes could be involved in controlling the life length of the mammals.

Therefore, BM derived SCs are a heterogeneous population of cells with HSC and TCSC, the morphological and functional characteristics of which are different from HSC. Their number among mononuclear cells (MNCs) is very low (approximately one cell per 1 000 – 10 000 marrow MNCs) within young mammals and might play a role in healing of small injuries [31, 32].

In severe injuries (like hart infarct or stroke) they have no possibility to reveal their full therapeutic potential. The allocation of these cells to the damaged areas depends on homing signals that maybe inefficient in the presence of some other cytokines or proteolytic enzymes that are released from damaged tissue associated leukocytes and macrophages. We can envision, for example that metalloproteinases released from inflammatory cells may degrade SDF-1 locally, and thus perturb homing of $CXCR4^+$ TCSC. There is possibility that these cells while "trapped" in BM are still in: "latent stage" – not fully functional and need the appropriate activation signals by up till now unknown factors [32–37].

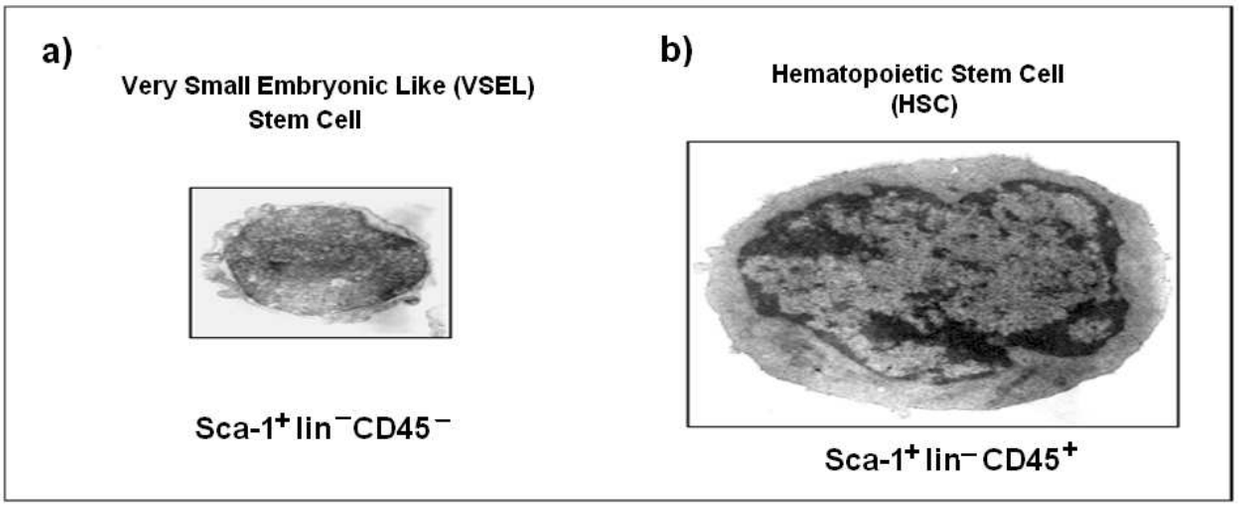
These cells also, at least in some cases could be attracted to the inflammatory areas, and if not properly incorporated into the damaged tissue they may transform and initiate tumor growth. Briefly, between the pools of TCSCs, there are probably those already committed to transdifferentiate into neural cells, or cells of tissues and organs other ten neural, but we still do not have the control over their tracking, homing and finally regenerative capacity in the given tissue, which is a fundamental prerequisite for successful regenerative therapy.

5. The concept of "very small embryonic-like" stem cells

In a discovery that has the potential to change the face of SC research, a University of Louisville scientist has identified cells in the adult body that seem to behave like embryonic SCs [38, 40–44]. The cells, drawn from adult BM, look like embryonic SCs and appear to mimic their ability to multiply and develop into other kinds of cells. The finding, presented the first time at the 47th Annual Meeting of the American Society of Hematology (ASH), was announced at the society's news conference. A study by Ratajczak's team published in the journal "Leukemia" was the first to identify a type of SC in adult marrow that acts differently than other BM derived

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SCs [38]. The newly identified cells – called "Very Small Embryonic-Like" (VSEL) SCs – have basically the same ultrastructure and protein markers as embryonic SCs [38]. Ratajczak and several other researchers from mentioned ASH meeting showed that VSEL SCs mobilize into the blood stream to help repair damaged tissue following a stroke [37]. In further research advance, Ratajczak's team also has grown VSEL cells in a lab and has stimulated them to change into nerve, heart and pancreas cells [40–42]. The differences in ultrastructure between HSC and VSELs are shown in Figure 1.



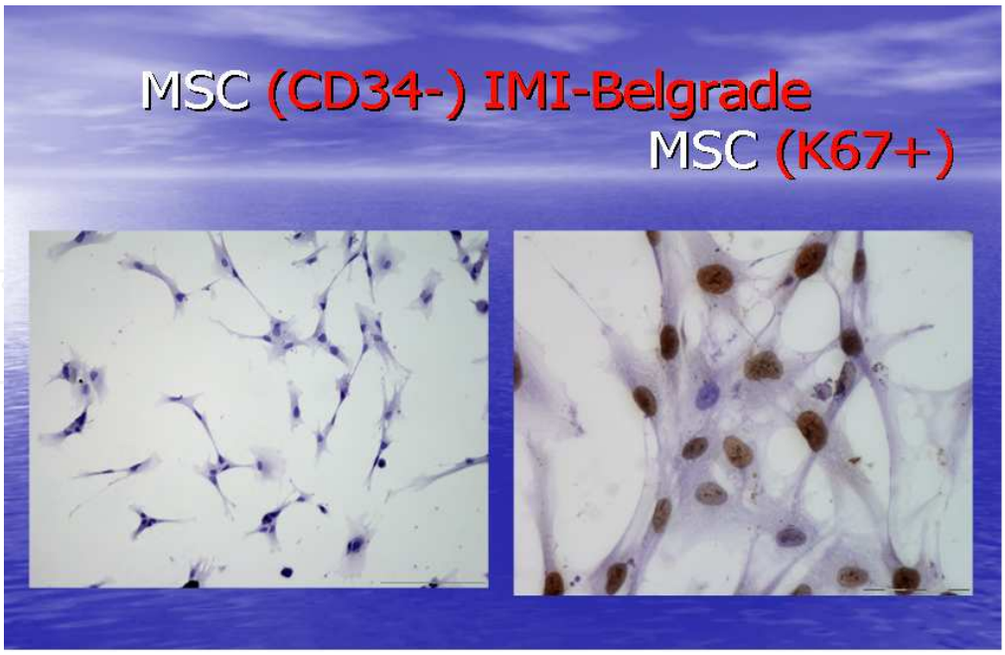
(For picture thanks to kind permission of M. Ratajczak)

Figure 1. Ultra-structural differences between mouse VSEL and HSC

Along with this new concept, there is a premise that in regenerative therapy done before, with HSCs (considered to have plasticity and multipotency) the VSELs were "contaminants" that actually contributed to positive regenerative clinical outcome, since they have those capabilities. This is an interesting concept which should be seriously considered in humans. However, since VSELs have been found in human UCB and BM first [37], and then used and applied with the patients [43–44] they seem to be of a critical importance for consideration of SC transplant choice based upon the phenotype and number of SCs aimed to be transplanted within a given clinical scenario.

6. The concept of mesenchymal stem cell – with dental pulp cells as an example

Many human tissues are the source of SCs responsible for tissue development and regeneration. Beside marrow (Bone Marrow Stromal Stem Cells – BMSCs), currently it is considered that dental pulp is practically the most approachable and the most important source of adult mesenchymal SCs [45–47] (Figure 2).



(With courtesy of V. Todorovic)

Figure 2. Mesenchymal stem cells

Within the last decade, several populations of SCs from dental pulp were isolated and characterized: a) Dental Pulp Stem Cells – DPSC; b) cells from Human Exfoliated Decidual teeth – SHED; and c) Immature Dental Pulp Cells – IDPC [46, 47]. These cells are of the ectomesenchymal origin, located in perivascular niche, highly proliferative, clonogenic, multipotent and similar to BMSCs. Within *in vitro* conditions, they can differentiate with certain intercellular differences toward odontoblasts, hondrocytes, osteoblasts, adipocytes, neuron/glial cells, smooth and skeletal muscle cells. Within *in vivo* conditions (after implantation) they show different potential for dentine formation, as well as osteogenesis; after transplant in mouse with compromised immune system, they make good grafts in different tissues, and are capable of migrating into the brain, where they survive a certain time while reaching neurogenic phenotype. DPSCs have immunomodulatory effect, as they can be involved into immune response during infection of dental pulp by NF-kB activation and by inhibiting T-cell proliferation, suggesting their immunosuppressive effect [47].

The future research should give us the complex data on the molecular and functional characteristics of dental pulp SCs, as well as differences between various populations of these cells. Such research would fundamentally contribute to the better knowledge on the dental pulp SCs, which is necessary due to their potential clinical application in *in vivo* cell transplant, tissue engineering, and gene therapy (*in vivo* or *ex vivo*). Actually, by the isolation of IDPCs, which are the most primitive, but also the most plastic (similar to embryonic SCs) they are opening the new perspectives in a potential therapeutic application of these cells not only in regeneration of dentine, otherwise also the regeneration of periodontal and "bone-junction" tissue of craniofacial region, as well as in the therapy of neurotrauma, myocardial infarction and other tissue damages [46, 47].

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7. Stem cell harvesting and *ex vivo* manipulation

For therapeutic use, SCs can be collected by: a) multiple aspirations from BM; b) harvesting from PB after mobilization (chemotherapy and/or growth factors – rHuG-CSF); and c) collection from UCB. Collected cells can be clinically applied (transplanted) immediately following harvesting (allogeneic setting) or after storage in frozen state or cryopreservation (autologous setting).

8. Bone marrow derived stem cells

Historically, BM was the first SC source for transplants. Cells were collected by multiple aspirations from the iliac crests, under sterile conditions, while the donor was generally anesthetized. The target volume of collected BM aspirate is 10 – 15 mL per kg of donor body mass (kgbm). In order to provide required number of total nucleated cells (TNCs) – that is TNC ≥ $3x10^8$/kgbm – around 200 aspirations are required (single aspirate volume = 2 – 5 mL) (Figure 3).



Figure 3. Stem cell collection from bone marrow

After collection, BM aspirate should be filtered in order to remove bone and lipid particles and cell aggregates. Anticoagulation is created using citrate solution and by using of the heparin diluted in saline (5 000 IU/500 mL) [2, 48–50].

BM aspirate volume – precisely red blood cell count or plasma quantity – reduction is required (processing), especially for ABO incompatible transplants. Depletion of T-cells in cell suspension is achieved using *ex vivo* purging (by immunomagnetic technique). These SC purification procedures (processing and purging) enable reduction of red cell for around 80 – 90% and depletion of T-cells with 3 – 4 Log_{10} [50].

9. Peripheral blood derived stem cells

The PB derived SC transplants are characterized by: a) less invasive cell collection; b) lack of the risks of general anesthesia; c) rapid hematopoietic reconstruction; d) low harvest volume (200 – 300 mL), and e) inferior transplant-related morbidity. Thus, the number of patients treated by PB derived SCs is ever increasing, especially in autologous transplant setting [6–8, 48–51].



Figure 4. Stem cell harvesting from peripheral blood

In steady state hematopoiesis SCs, that is $CD34^+$ cells are in very low proportion (0.01% to 0.1% compared to MNCs) in PB, but they can be mobilized from BM. Allogeneic donors are given rHuG-CSF 5 (10 μg/kgbm per day). The count of $CD34^+$ cells in the circulation begins to rise

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after 3rd day, and peaks on the 5th day of rHuG-CSF administration. In autologous setting, patients are given higher rHuG-CSF dose (12-16 μg/kgbm or more daily) combined with chemotherapy [8, 48–51].

In allogeneic setting, the first SC collection is performed typically on the 5th day (Figure 4.). The optimized timing of an autologous SC harvesting is more complex and controversial. The leukocyte count commonly does not correlate with the circulating $CD34^+$ number. The count of circulating $CD34^+$ cells evidently correlate with the superior $CD34^+$ yield in harvest. Generally, at PB $CD34^+$ count ≅ 10/μL, expected SC yield ≅ $1x10^6$ per kgbm. It is also presented that for a $CD34^+$ ≥ 20-40/μL of PB the possibility of the $CD34^+$ ≥ $2.5x10^6$ per kgbm is 15% using one standard apheresis, and 60% after one large-volume SC harvesting [6–8, 49].

The target CD34+ cells should be $330x10^6$ per unit or ≥ $2\text{-}4x10^6$/kgbm of the recipient in order to expect successful transplant. Recent data support a benefit associated with greater $CD34^+$ yield (≥ $5x10^6$/kgbm) compared to the minimum required cell quantity for engraftment (≥ 1 x 10^6/kgbm) in autologous setting [20]. Finally, results obtained in our SC transplant center confirmed that large-volume apheresis is efficient ($CD34^+$ ≥ $5x10^6$/kgbm) if the circulating $CD34^+$ count was around 40-60/μL after mobilizing regiment [8].

10. Umbilical cord blood derived stem cells

Patient's request for SCs have only in ≤ 30% (related) and ≤ 85% (unrelated) possibility of finding an adult allogeneic donor [20]. Because of the limited availability of donors, attention has turned to alternative sources of HLA-typed SCs. In recent years, UCB has emerged as a feasible alternative source of transplantable $CD34^+$ cells for allogeneic transplant, mainly in patients who lack HLA-matched donors of BM or PB derived SCs [9–1 2].

SCs obtained from the UCB immediately after birth are usually referred to as neonatal SCs. These cells are less mature than those in BM. The advantage of the use of UCB is painless and non–invasive collection. UCB has advantage that – despite its high content of immune cells – it does not produce severe GvHD. Precisely, the "naive" nature of UCB lymphocytes permits the use of partly HLA-mismatched grafts without higher risk for severe GvHD relative to BM transplant from a full matched unrelated donor. Thus, UCB grafts do not need to be as "rigorously" matched to a recipient as BM or PB grafts [10–12].

On the contrary, the major disadvantage of this cell source is the limited number of SCs. UCB volume is typically 80 – 200 mL, with a TNC count ≈$1x10^9$ and approximately $CD34^+$ count ≈$3x10^6$ per unit. Thus, UCB is an accepted cell source for pediatric patients and for whom a matched unrelated BM or PB cell donor is unavailable. However, a higher risk of graft failure was noticed in children weighing ≥ 45 kg. Since the number of SCs in UCB is limited and the collection can occur only in a single occasion – its use in adult patients can be more problematic. Finally, since SCs in the UCB are "more primitive", the engraftment process takes longer with UCB, leaving the patient vulnerable to posttransplant infections or bleeding. However, "more primitive" SCs in UCB have the potential to give rise to non-hematopoietic cells (myocardial, neural and endothelial cells, etc) by transdifferentiation [2, 9–12].

11. Stem cell cryopreservation practice

Efficient transplant program requires both, high-quality harvesting and cryopreservation techniques for obtaining adequate yield and recovery of the SCs. In practice, SC cryopreservation consists of the following steps: a) aspirate processing; b) equilibration (cell exposure to cryoprotective agent) and freezing; c) storage at temperature –80±5°C (mechanical freezer), at –140±5°C (mechanical freezer or steam of nitrogen) or at -196°C (liquid nitrogen); and d) cell thawing in a water bath at 37±3°C. There are several cryopreservation protocols, using primarily DMSO in autologous plasma. The optimal cooling velocity in controlled-rate cryopreservation setting is –1°C. The transition from liquid to solid phase is also critical period – because of released fusion heat – since a significant reduction in cell recovery and viability has been observed when this period is prolonged. The compensation of the released fusion heat is required, using elevated cooling rate (–2 °C per minute) during transition period. Finally, there is data showing that uncontrolled-rate freezing is also useful in SC cryopreservation [48–52].

Cryopreserved SCs are thawed rapidly in a water bath at 38±2°C at the patient's bedside and infused immediately through a central vein catheter. Generally, patients tolerate the infusion of unprocessed SCs well, with no side effects. The grade of the potential reinfusion–related toxicity is associated with total DMSO quantity in the thawed cell concentrate. Alternatively, cryoprotectant can be removed by washing after thawing, but this procedure results in substantial cell loss.

12. Conventional stem cell transplants – A synopsis of the clinical practice

Generally, SC transplants include the use of high-dose chemotherapy in order to obtain disease eradication and (re)infusion (allogeneic transplant or autologous SC support) of cells collected to get hematopoietic and clinical renewal. SC transplant with reduced-intensity conditioning (RIC) can be offered to patients who are disqualified for high-dose conditioning because of their age or comorbidities. Malignant hematological diseases and some immune-mediated disorders (Table 1) are the most common indication of this therapeutic approach using SCs [2, 50].

The efficacy of transplants depends on the type of disease, its stage and sensitivity for chemotherapy, patients' age and general condition, as well as degree of the HLA-matching. In general, survival rates are around 30 – 60% for otherwise fatal diseases. Details of the SC clinical use – that is optimized treatment timing and efficacy, peritransplant complications, etc. – of the transplants in presented hematological disorders will not discussed in this paper.

Briefly, autoimmune diseases, which do not respond to standard immunosuppression, could benefit from immunoablative therapy. The idea of treatment of immune-mediated disorders (e.g. multiple sclerosis) by autologous SC transplant is based on the hypothesis that immunoablative treatment can destroy the patients "anti-self-lymphocytes" (i.e. an "immune-resetting" process). The beneficial immunomodulating effect of allogeneic SC transplant in

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therapy of hematological malignancies has long been known, but only recently have systems been developed to separate the GvL effect from GvHD. Using donor-specific lymphocytes, the best results were obtained in chronic myelogenous leukemia. Moderate successes have been reported in relapsing acute myeloid leukemia, myelodysplastic syndrome, multiple myeloma and some responses were obtained for acute lymphoid leukemia [2, 8, 50].

BM malignant or dysplastic disorders
Leukemias
Hodgkin's and non-Hodgkin's lymphoma
Multiple myeloma
Myelodysplastic/myeloproliferative disorders
Benign immune mediated disorders
Severe combined immunodeficiency disease
Marrow failure syndromes
Severe aplastic anemia
Autoimmune disorders
Thalassemia
Congenital Immune deficiencies
Solid tumors
Breast cancer
Ovarian cancer
Testicular cancer
Wilm's tumor
Neuroblastoma
Rhabdomyosarcoma
Ewing sarcoma

Table 1. Current indications and relative suggestions for SC transplant

Although SC transplant-related mortality and morbidity have reduced, SC transplants continue to pose numerous potential complications. The most frequent complications are even now engrafting failure, virus or opportunistic infections and acute or chronic GvHD. Less toxic transplants, in the form of non-myeloablative conditioning regimens, are being actively investigated, with the promise of expanding indications for allogeneic transplant. In addition, SC transplant with RIC can be offered to patients who are disqualified for high-dose conditioning because of their age or comorbidities. A careful proactive assessment to identify, treat, and, hopefully, prevent adverse events is essential to a successful transplant [2].

We have previously analyzed our results of PB vs. BM derived SC transplants based on the hematopoietic reconstitution. Transplants were used for the treatment of patients with severe aplastic anemia, acute lymphoblastic leukemia, acute non–lymphoblastic leukemia, chronic myeloid leukemia, multiple myeloma, Hodgkin's and non–Hodgkin's lymphoma, breast and ovarian cancer, extragonadal non–seminal germ cell tumor, and severe multiple sclerosis. The $CD34^+$ yields for allogeneic and autologous transplants were eminent: $16.7\pm9.8 \times 10^6$/kgbm and $11.8\pm6.1 \times 10^6$/kgbm, respectively [8, 26, 27]. A typical histogram with high–level of the $CD34^+$ ratio in obtained PB harvest is presented in Figure 5.

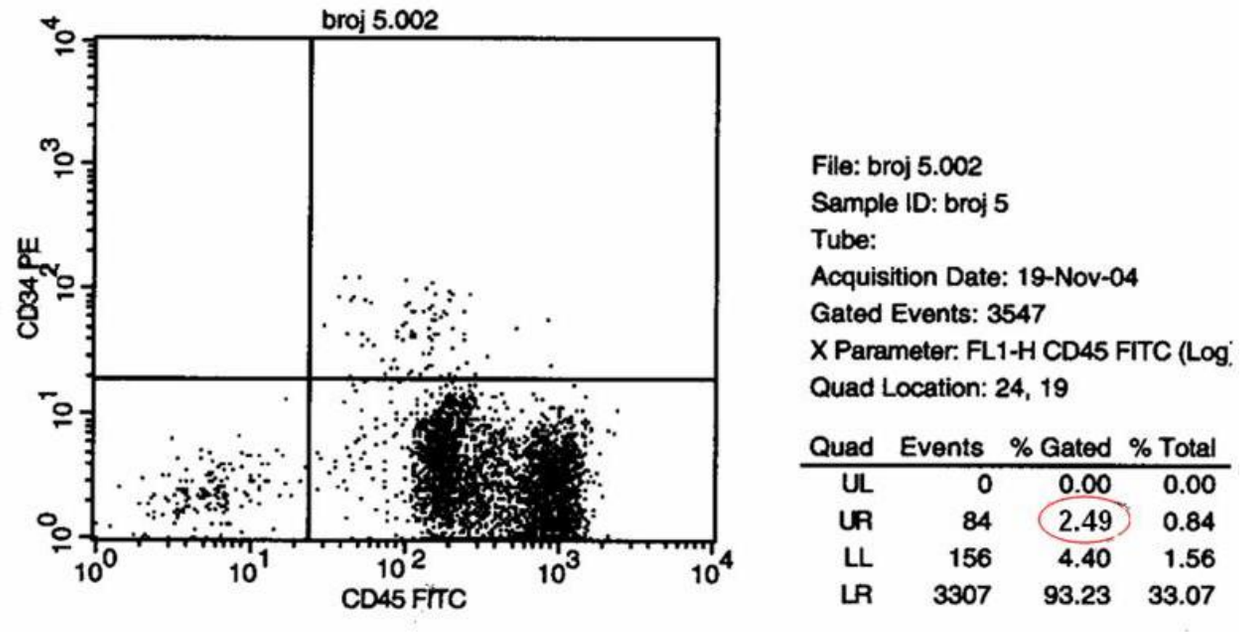


Figure 5. The ratio of the $CD45/CD34^+$ cells in peripheral blood harvest

For autologous SC transplants, the use of the best freezing process and choice of the most appropriate cryoprotective agent is required (optimized cryobiosystem). Nowadays a variety of protocols are used in blood–derived cell freezing practice. Generally, microprocessor–controlled (controlled–rate) freezing is more efficient than uncontrolled–rate (without programmed cooling) procedure due to better cell recovery. Our earlier results obtained for cryopreserved bone marrow cells and peripheral blood mononuclear cells were in agreement with these findings [8, 52]. These results imply a different "cryobiological request" of MRA cells in comparison with the mature progenitors. Moreover, our clinical studies showed that therapeutic use of the SCs – cryopreserved by our own controlled-rate system resulted with high cell recovery (91%) and rapid posttransplant hematopoietic reconstitution – on the 11^{th} day in average [2, 8].

We have also investigated SC–harvesting protocols with optimized cell source, collection time–point and processed blood volume, CD34–threshold dose (calculed by ideal body mass), as well as immature ($CD34^+/CD33^-$, $CD34^+/CD38^-$, $CD34^+/DR^-$, $CD34^+/CD90^+$) vs. mature ($CD34^+/CD33^+$, $CD34^+/CD38^+$, $CD34^+/DR^+$, $CD34^+/CD90^-$) CD34–subset ratio in harvest [8, 26, 28, 50].

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Several data related to our immature vs. mature $CD34^-$subset investigations of cells collected from different sources are presented in Table 2.

	PB–SCs I	PB–SCs II	BM–SCs
CD34 PE / CD90 FITC [%]	1,72±1,47	1,25±0,82	2,72±2,06
CD34 PE / CD38 FITC [%]	2,02±1,18	2,02±1,18	2,3±1,16
CD34 PE / HLA–DR FITC [%]	2,01±0,92	2,0±0,92	2,0±0,88
CD34 PE / CD33 FITC [%]	1,90±1,23	1,9±1,23	2,75±1,12

PB–SCs I = stem cell collected from peripheral blood after mobilization with chemotherapy and rHuG–CSF; PB–SCs II = stem cell collected from peripheral blood after mobilization with rHuG–CSF alone; BM–SCs = stem cell collected from bone marrow.

Table 2. Ratio of CD34 cell markers using double staining

Finally, we found that the use of large volume vs. conventional (repetitive) apheresis resulted in improved $CD34^+$ yield and viability (7–AAD flow cytometric assay) [2, 8, 50]. The harvesting of higher ratio of immature vs. mature CD34–subsets correlated with BM repopulation ability (complete and long–term engraftment) and rapid hematopoietic reconstitution, as well as superior organ repair or SC regenerative potential.

SCs are considered optimal targets for gene transduction due to their ability to renew themselves and differentiate into progeny cells and generate a self-perpetuating cell population that contains the transduced gene for the lifetime of the patient. Specific diseases that could be candidates for SC gene therapy include thalassemia, sickle cell anemia, Fanconi anemia, severe combined immune deficiency secondary to adenosine deaminase deficiency or purine nucleoside phosphorylase deficiency, chronic granulomatous disease, leukocyte adhesion deficiency, Gaucher's disease, and a variety of other metabolic/storage deficiencies. UCB derived SCs potentially could be used to correct genetic deficiencies at birth after successful gene transduction and autologous transplant [2, 50].

13. Stem cells in regenerative medicine – A rapid consideration

Cardiac repair following SC application.The occurrence of heart failure following acute myocardial infarction – during the hospital stay and during the next few months or years – is high (up to 50%). Patients' mortality with heart failure after infarction is also considerable. The left ventricle dilatation occurs in even approximately one third of the patients, reperfused effectively with primary angioplasty. The incidence of heart failure after infarction has increased and mortality decreased with better reperfusion therapy [2, 3, 28]. Consequently, it is imperative to develop a curative approach to prevent of myocardium remodeling. The SC therapy is a new and promising manner of an infarcted heart healing.

It is generally accepted that adult SCs from different tissues may transdifferentiate in special situations into cardiomyocytes or endothelial cells. In addition, the presence of an important quantity of cardiac cells is confirmed in proliferative state in peri-infarction region. The first source of these "regenerative cells" is maybe cardiac SCs – which are in the inactive stage in intact (undamaged) myocardium – but following infarction they differentiate into cardiomyocytes, smooth muscle cells and endothelial cells [2, 3]. The next discovery is that for the period of infarction, myocardial ischemia initiates release of some cytokines, growth-factors and chemokines – which induce SC mobilization from other niches and their homing into the damaged myocardium. The knowledge of these processes is important because the treatment efficacy depends on artificial *ex vivo* and/or *in vivo* intensification of some "steps" in order to make regenerative process more beneficial.

The exact mechanism by which SCs create a protective effect resulting in tissue/organ repair and heart function improvement is also a matter of debate. A number of possible mechanisms have been proposed: a) SC transdifferentiation into cells of other lineages (cardiomyocytes or endothelial cells) resulting in formation of new tissue; b) mobilization of tissue specific SCs from the BM that home to the damaged tissue and participate in tissue regeneration; c) fusion of the SCs with cells of the target tissue giving rise to new cells; and d) creation of a milieu (perhaps by releasing growth factors) that enhances regeneration of endogenous cells [2, 3, 50].

At present, BM is the most frequent source of cells used for clinical cardiac repair. It contains a complex mixture of progenitor cells – including SCs; so-called side population (SP) cells, which account for most if not all long-term self-renewal and reconstitute the full panoply of hematopoietic lineages after single-cell grafting; a subset of mesenchymal or stromal cells (MSCs), which are already defined; multipotent adult non-hematopoietic progenitor cells (MAPCs – for example, VSEL cells), which can differentiate into all possible lineages, and a fraction of TCSC discovered recently by Ratajczak et coworkers [2, 38–44]. These TCSCs circulate at the highest level and thus accumulate in BM during rapid body growth and become a reserve pool of SCs for tissue/organ regeneration. They are chemo-attracted from PB to injured organs by signaling proteins, such as stromal cell-derived factor-1, which become highly expressed in damaged heart tissue. For therapeutic purposes, marrow is aspirated, the entire MNC fraction is obtained – a mixed combination of mentioned cells – or specific subpopulations are purified and isolated cells are injected into the heart without need of further *ex vivo* manipulation/expansion.

In conclusion, current challenges for cell-based therapy in cardiac repair include identifying the origins of the novel cardiac SCs found inside heart, pinpointing biologically active cells from BM and other cell populations, optimizing cell mobilization and homing, increase of survival of grafted SCs, and exploiting cell therapy as a platform for secretor signals. Thus, we need a lot of basic research and randomized clinical trials to define the exact role of this probably revolutionary therapy for ischemic heart disease.

Application of SCs for liver and pancreas regeneration. The growing donor organ shortage requires consideration of alternative emerging technologies. Regenerative medicine may offer novel strategies to treat patients with end-stage or severe organ failure.

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The final purpose of SC therapy, organ repopulation strategies and tissue engineering is to regenerate tissues/organs or to produce new grafts/organs for transplant. With the expansion of complete organ "decellularization" methods the equation of organ shortage could be radically altered in the future. Decellularized organs provide the ideal transplantable platform with all the essential microstructure and extra-cellular signals for cell connection (homing), transdifferentiation, tissue vascularization, etc. Novel systems to re-engineer organs may have key connotations for the fields of regenerative biology and ultimately organ transplant [2, 50].

Currently available β-cell replacement therapies for patients with Type 1 diabetes (T1D), including islet and pancreas transplant, are largely successful in restoring normal glucose metabolism. However, there are data concerning the use of SCs to generate β-cells for islet transplant, indicating the need for improved protocols for their derivation and full maturation. Researchers also considered evidence indicating that adult SCs may affect islet transplant by improving the viability of engrafted islets and controlling immune-related reactions to islet antigens. A novel SC-based applications or regeneration-type approaches include stimulation of endogenous regenerative mechanisms or inducing reprogramming of non-β cells into β cells. Because these strategies would finally generate allogeneic or syngeneic β cells, the control of alloimmunity or autoimmunity in addition to replacing lost β cells will be of the greatest importance [2, 50].

For the SC treatment of our patients with liver failure (n = 8) and T1D (n = 4), cells were harvested from PB following mobilization (rHuG-CSF 10μg/day; 5 days). The mean volume of processed blood was 15.2±1.6 L (ratio: 12.8 – 18.4 L). The total count of MNC and $CD34^+$ collected cells were $6.4\pm3.1x0^9$ and $1.6\pm08x10^7$, respectively. Cells were applied after immunomagnetic selection and *ex vivo* transdifferentiation and expansion across catheter [2, 50].

The use of SCs in neurology/neurosurgery. Alzheimer's, Parkinson's and Huntington's diseases, amyotrophic lateral sclerosis (ALS), and Friedreich's ataxia are the most common human neurodegenerative diseases – pathologically characterized by a progressive and specific loss of certain neuronal populations. Currently there are no effective clinical therapies for many of these diseases. The recently acquired ability to reprogram human adult somatic cells to "induced pluripotent SCs" (iPSCs) in culture may provide a powerful tool for *in vitro* neurodegenerative disease modeling and an unlimited source for cell replacement therapy. Reprogramming of somatic cells into iPSCs ushered in a new era of regenerative medicine. Human iPSCs give potent new approaches for disease modeling, drug testing, developmental studies, and therapeutic applications.

We earlier largely described the specific therapeutic actions and clinical use of SCs in neurology/neurosurgery [2]. Cerebral tumors, stroke, neurodegenerative diseases, brain and spinal injuries are used as examples with different limitations and possibilities to be approached with adult SC and/or different regenerative treatments. It is clear, that despite a spectrum of successful approaches, there are current limitations in this field of therapeutic interventions, which makes the research more intriguing and opens the new avenues for the development of novel concepts, their future prove, and possible application.

Finally, in our center SC are applied the first time in the treatment of ALS female patients (intratecal application of non-manipulated BM derived cells; two repeated treatments) and another female patient after brain infarction (intra-arterial cell injection using percutaneous catheter). Logically, preliminary or particularly definitive conclusions can only be drawn from larger, randomized, controlled clinical trials.

14. The treatment of large myocardial infarction by intracoronary applied rHuG-CSF facilitated BM derived SCs – Our experience

14.1. Introduction

Intracoronary autologous, BM derived SC transplant for the treatment of myocardial infarction went through the three important steps during the last decade. In the first step, small non-randomized trials of Strauer [53] and TOPCARE study [54], established the basic methodology of SC harvesting, cell processing and intracoronary delivery and confirmed the safety and regenerative potential of SCs for the improvement of myocardial viability and function after infarction.

The next step representing two landmark studies has brought controversial results. The REPAIR-AMI [55] has showed that intracoronary delivery of BM derived SCs led to the improvement of six months global ejection fraction and lowers the major cardiac adverse events. On the contrary, the ASTAMI trial [56] has failed to prove any benefit of early intracoronary application of BM derived SCs to the global ejection fraction and left ventricle remodeling measured by magnetic resonance imaging at baseline and after four months of anterior infarction. However, those studies suggested several important issues. The first of all were that choice of patients with more severe damaged myocardium and the delayed SC delivery for at least five days after infarction resulted to better results of SC regenerative capacity. The second key conclusion is that SC processing process might be essential for successful SC therapy after myocardial infarction.

In the third step, two relatively large randomized studies tried to define the efficacy of different SC population for treatment of myocardial infarction. The REGENT study [57] used selection of $CD34^+$/CD-$CXC4^+$ cells, and the HEBE trial [58] examine selection of PB derived MNCs and compare the results with two control groups, non-selected BM origin MNCs and controls. These trials didn't show any usefulness of cell selection and suggested again that patients with more damaged myocardium had better improvement with intracoronary SC delivery. On the other hand, our study has showed that there is a limit for the amount of myocardial necrosis in which we can achieve improvement of myocardial function after intracoronary SC delivery and the patients with the huge loss of myocardium has no any benefit from the cell therapy [26–28]. However, improvement of global and regional left ventricle function was modest and faraway from the expected in all studies. The next steps in SC therapy are *ex vivo* expansion of the number and regenerative capacity of harvested SCs, *in vivo* facilitation of that, and improvement of methods of SC delivery.

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In our Center for regenerative medicine, total of 60 patients were treated by non-manipulated or *ex vivo* cytokine stimulated BM derived cells (collected in steady state hematopoiesis or after priming of the marrow). Cells were applied across percutaneous catheter intracoronarly or directly into the myocardium (transpericardial approach). In the most recent stage, we investigated the effects of rHuG-CSF facilitated BM (primed BM) SC therapy on improvement of the global and regional function of left ventricle after large myocardial infarction.

14.2. Methodology

The main inclusion criteria for the enrollment in the study are: patients with the first ST segment elevation myocardial infarction; age younger than 71 years old; successful percutaneous coronary intervention on the infarction artery inside the 24 hours from the onset of pain and with the left ventricle ejection fraction lower than 41% at the fifth day estimated by the transthoracic echocardiography. The main exclusion criteria are the presence of other serious illness, any pre-infarction significant damage of the heart, allergy to aspirin and resistance to clopidogrel, and the presence of the symptoms and signs of heart failure five days after infarction. The local Ethical Committee approved the study and all patients were given written informed consent.

Pre-transplant examination. At the fifth day of infarction, global and regional left ventricle systolic function together with the end-diastolic and end-systolic volumes are measured by the transthoracic echocardiography. Infarction size is estimated by the Technetium-sestamibi myocardial scintigraphy between the 5-8 days from the infarction.

Stem cell harvesting and application. The day before BM harvest, patients receive 5 – 10 μg/kgbm of rHuG-CSF. Between 7 – 12 days from the infarction in the general anesthesia 300 mL of the BM is harvested from the posterior iliac crests. After that BM was filtered and processed to the final volume of 30 – 50 mL of concentrated mono-nuclear cell suspension. Boluses of 10 mL are injected through the diagnostic catheter into the infarction related coronary artery. Patients with rHuG-CSF facilitated SC therapy received $18.4x10^8$ of MNCs and patients without rHuG-CSF received $7.9x10^8$ of the MNCs.

Control groups. There are two control groups with the same inclusion and exclusion criteria. The first are patients who did not submit to any SC procedure. And the second represents the patients who were treated with the autologous intracoronary, BM derived SC therapy without rHuG-CSF.

The follow-up. Clinical examination is scheduled for the one, fourth and sixth months after infarction and every 6 months after that. Echocardiography measurement of global and left ventricle ejection fraction and volumes is planned after 4 months and every year after infarction. Myocardial scintigraphy is planned after 4 months and after two years from the infarction.

End points. The main end points are comparison of the 4 months and 2 years change in left ventricle ejection fraction, volumes and infarction size between three groups.

14.3. Results

The baseline characteristics of three groups of patients were similar except that control group were slightly older than patients in both SC groups and have more often multivessel disease. Gender and risk factors distribution were similar between groups. There is also no difference in ischemic time in three groups. Baseline global left ventricle ejection fraction and infarction size were similar in all three groups. However, both end-diastolic and end-systolic cardiac indices were lower in patients with rHuG-CSF facilitated SC therapy.

After 4 months left ventricle ejection fraction has improved in group of patients treated with SCs and in control group but did not reach statistical significance in the group treated with rHuG-CSF facilitated SC therapy because of small number of patients in that group (Table 1). Infarction size has the same pattern (Table 3). End-diastolic and end-systolic volumes increased in all groups but also did not get to significant statistical difference in the rHuG-CSF facilitated SC group.

Parameters	SC therapy in AMI n=19	G-CSF facilitated SC therapy in AMI n=5	Control group n=17	p
Infarction size at baseline LV%±SD	28,4±11,3	35,6±8,0	31,4±12,8	ns
Infarction size after 6 months LV%±SD	25.2±12.6	25.2±8.6	27.9±10.7	ns
p	0.001	0.068	0.001	
LVEF at baseline % ± SD	32,9±4,1	36,4±3,0	34,3±5,2	ns
LVEF after 4 months % ± SD	37,0±9,0	43,8±3,0	36,9±8,2	0,01
p	0.004	0.313	0.004	
EDVCI at baseline ml/m^2 ± SD	68,3±11,3	46,1±10,0	67,8±17,6	0,01
EDVCI after 4 months ml/m^2 ± SD	75,7±15,7	54,3±6,1	73,8±20,4	0,053
p	0.024	0.161	0.004	
ESVCI at baseline ml/m^2 ± SD	44,8±9,8	28,0±4,4	45,0±15,1	0.02
ESVCI at 4 months ml/m^2 ± SD	47,8±14,3	30,3±2,6	47,5±17,6	0,07
p	0.001	0.142	0.002	

Table 3. Infarction size end left ventricle systolic function and volumes at baseline and after 4 months.

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Difference between baseline and 4-months infarction size is significant only in patients with rHuG-CSF facilitated SC therapy (Figure 6). There was no significant difference between the change of LVEF at baseline and after 4 months (Figure 6).

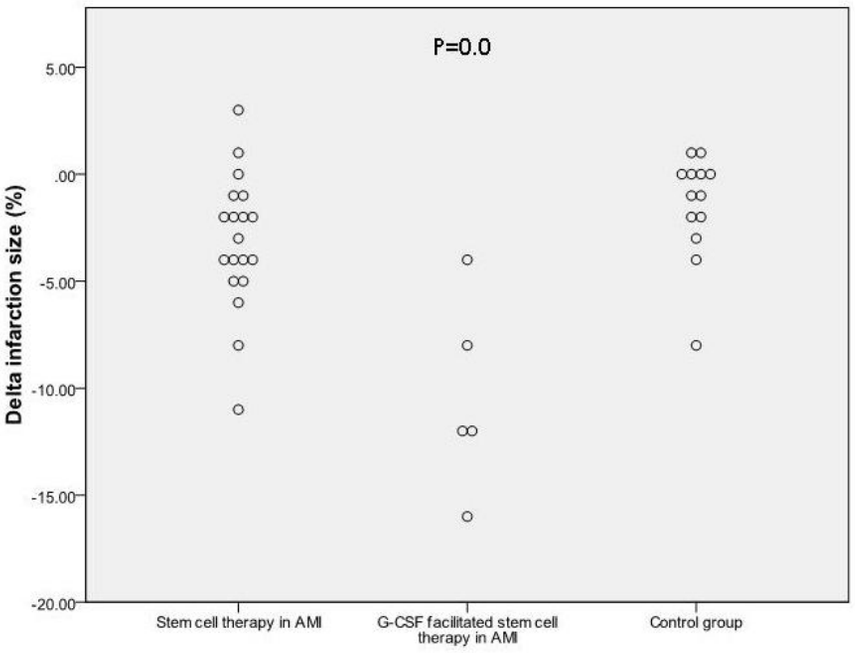


Figure 6. Change in infarction size between baseline and 4-months among three groups of patients

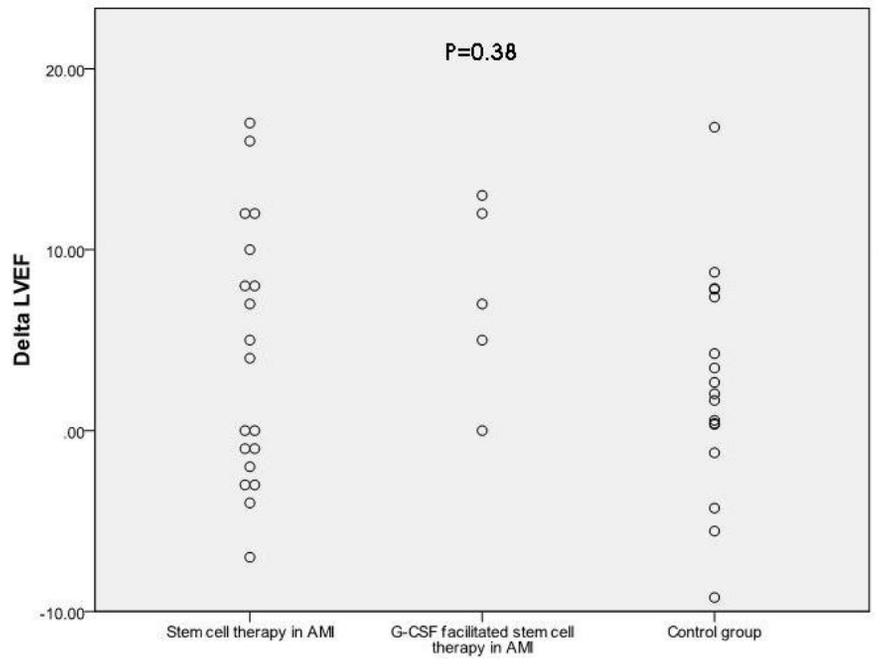


Figure 7. Change in left ventricle ejection fraction: baseline vs. 4-months among three groups of patients

15. Conclusion

Our preliminary results have shown that rHuG-CSF facilitated autologous, intracoronary SC transfer was safe; between two-three times higher number of MNCs were given and there was a trend toward larger increase of 4-months ejection fraction and greater decrease of the infarction size than the control groups. Any procedure that increases the left ventricle ejection fraction for more than 5% after a several months follow-up would be of great clinical and economic value. Autologous BM derived, intracoronary SC transfer in the second week of large myocardial infarction very probably improved the global left ventricle ejection fraction by 3 – 5% although results of the published trial are controversial. Granulocyte colony stimulating factor given alone for several days after myocardial infarction did not improve significantly global ejection fraction in the several trials, but it seems that its early application in patients with larger infarction could be useful. Further investigation is needed for the justification of rHuG-CSF facilitated, BM derived SC therapy in the early phase of acute ST elevation myocardial infarction.

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