

The Therapeutic Potential of Stimulating Endogenous Stem Cell Mobilization

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1. Introduction

The past decade has seen a fast and extensive development of various therapies and treatment protocols based on Adult Stem Cells (ASC) and their application to various diseases. While some of these treatment protocols have been well documented in the scientific literature and used in well controlled clinical set ups, others have been developed and are being used by a growing numbers of clinics throughout the world, without thorough documentation though nevertheless with good clinical care and with the reports of very compelling results.

Despite the wide variety of methods, the general procedure guiding these various protocols follows a series of common steps. The first step is the isolation of stem cells from a source. For the purpose of banking or clinical application, stem cells can be isolated from a variety of sources including umbilical cord (Can and Balci, 2011; Zhang et al., 2011), adipose tissue-derived stem cells (Insausti et al., 2011; Zachar et al., 2011), peripheral blood stem cells (Kolbe et al., 2010; Hofmann et al., 2009), amniotic and placental stem cells (Klein and Fauza, 2011; Tsagias et al., 2011), dental pulp stem cells (Gronthos et al., 2011; Tirino et al., 2011), olfactory stem cells (Chen et al., 2006; Viktorov et al., 2008), and even human limbal epithelial stem cells (Vasania et al., 2011).

The second step is proliferation. This is not a necessary step with regard to stem cell function, however the small number of stem cells present in one umbilical cord, one placenta, one blood sample, one liposuction or one dental pulp makes clinical application difficult without the ability to expand the harvested stem cells. Methods to expand embryonic stem cells have been developed more than a decade ago, however it is only a few years ago that methods to significantly expand ASC have been developed, leading to an expansion of the stem cell banking market and greater clinical application (Ivanovic et al., 2011; Dos Santos et al., 2011; Pineault et al., 2011).

The third step is pre-conditioning or treatment to trigger commitment of the stem cells into a specific cellular lineage. For example, stem cells can be led to differentiate into dopamine-producing neuron by an exposure to a cocktail containing sonic hedgehog (SHH), fibroblast growth factor 8 (FGF8), and basic fibroblast growth factor (bFGF) (Trzaska and Rameshwar, 2011; Wang et al., 2011) or into neurons responding to multiple neurotransmitters by a simple exposure to retinoic acid and other growth factors (Greco et al., 2008). Likewise, stem cells can be guided to differentiate in cardiomyocytes by exposure to a cocktail containing transforming growth factor-beta(1), bone morphogenetic protein-4, activin A, vascular endothelial growth factor (VEGF), insulin-like growth factor-1, fibroblast growth factor-2, Epidermal growth factor (EGF), and interleukin-6 (Behfar et al., 2010; Behfar et al., 2008). Pre-conditioning with these cytokines can also enhance the formation of gap junction and improve therapeutic efficacy (Hahn et al., 2008). Exposure of stem cells to a cocktail containing insulin, transferrin, selenium and the GLP-1 (glucagon-like peptide-1) analogue exendin-4 leads to the formation of insulin-producing pancreatic cells (Docherty, 2009; Chandra et al., 2009). Various cocktails have been shown to trigger the differentiation of mesenchymal stem cells in into a wide variety of cell types (Snykers et al., 2011; Arufe et al., 2009; Keilhoff et al., 2006). Nevertheless, this pre-conditioning step is not essential since ASC will naturally differentiate into the cell type with which they find themselves, upon contact with cellular debris or cell marker specific to that cell type. For example, as they migrate into the heart, stem cells can be triggered to differentiate into cardiomyocytes (Orlic et al., 2001), or into keratinocytes and skin appendages, insulin-producing pancreatic cells or hepatocytes as they respectively migrate in a skin wound (Zhang and Fu, 2008), the pancreas (Hasegawa et al., 2007) or the liver (Theise et al., 2000).

The third and final step is the injection of stem cells into the target organ, in the main artery leading to the target organ or in the bloodstream from where a number of them will migrate on their own to the affected organ. In the case of a heart attack for example, stem cells can be injected in coronary artery (Wollert et al., 2004) or directly in the border zone of the infarct (Stamm et al., 2003; Tse et al., 2003). Treatment efficacy can vary significantly with the various methods of injection. For the treatment of acute myocardial infarction, injection in the border zone of the infarct seems to yield the best results, followed by intracoronary and intravenous injection, respectively (Karra and Wu, 2008). For the treatment of spinal cord injury, injection directly in the lesion or in the cerebrospinal fluid seems far superior to intravenous injection (Lima et al., 2010), yet very compelling cases have been documented following intravenous stem cell injection or simple bone marrow stem cell mobilization (see Section 4). For the treatment of diabetes however, intravenous injection seems to yield better results than stem cells transplantation directly into the pancreas (Hasegawa et al., 2007).

In this multistep procedure in which each step can be accomplished according to a wide variety of protocols and methods, it remains that peripheral blood stem cells (PBSC) can, without expansion, pre-conditioning or injection reach various target organs and participate to the process of tissue repair. This observation has led a number of researchers to look at the therapeutic potential of simply stimulating Endogenous Bone Marrow Stem Cell Mobilization (ESCM). This chapter will look in detail into the clinical and therapeutic potential of ESCM by describing its physiological basis, by reviewing the existing literature on the clinical application of ESCM and by presenting a few clinical cases.

2. The repair system of the body

For ESCM to have any clinical relevance, the demonstration must be made that one of the natural roles of stem cells in the body is to participate to tissue repair in cases of injury or degenerative diseases. Therefore, the clinical relevance of mobilizing endogenous bone marrow-derived stem cells (BMSC) would be to increase the number of circulating stem cells available to migrate into affected tissues and contribute to tissue repair. For this phenomenon to be natural: 1) the body must have a mechanism that triggers BMSC mobilization after an injury; 2) BMSC must traffic in the blood and be recruited by the injured tissue; 3) in the injured tissue BMSC must proliferate and 4) a mechanism must exist to trigger the differentiation of BMSC into cells of that tissue.

2.1 Signaling for mobilization

The most common compound known to naturally stimulate BMSC mobilization is Granulocyte-Colony Stimulating Factor (G-CSF). Discovered in 1985 by Welte et al., G-CSF is a cytokine secreted by various tissues that stimulates the proliferation, differentiation and function of neutrophil precursors and mature neutrophils. But G-CSF was also shown to stimulate BMSC mobilization (Petit et al., 2003; Cottler-Fox et al., 2003), making it a common tool in protocols of stem cell apheresis for the purpose of cryopreservation and stem cell transplant (Gordon et al., 2008; Croop et al., 2001).

Given the vital importance of the heart and the fact that cardiovascular diseases are a leading cause of death in the world, much of the clinical stem cell research has focused its efforts on the role of stem cells in cardiac repair taking place after acute myocardial infarction (AMI). A number of studies have revealed the sequence of events taking place after AMI. A few hours after AMI, the cardiac tissue releases or causes to release G-CSF (Leone et al., 2006). As its concentration slowly increases in the bloodstream, G-CSF triggers the release of stem cells from the bone marrow, increasing the number of PBSC which peaks at around 4-7 days after AMI (Shintani et al., 2001; Leone et al., 2006). It is worth mentioning that the serum level of G-CSF and the number of PBSC are also increased in cases of chronic angina (Leone et al., 2006). Similar stem cell mobilization and increase in PBSC have been documented following skeletal muscle injury (Stout et al., 2007).

Other chemokines such as interleukine-8 (IL-8), Stromal-Derived Factor-1 (SDF-1), Stem Cell Factor (SCF), Gro β , and vascular endothelial factor (VEGF) have been shown to trigger BMSC mobilization (King et al., 2001; Lapidot & Petit, 2002; Fukuda et al., 2007; Lapidot et al., 2009). Contrary to G-CSF and SCF, which lead to a slow increase in the number of PBSC over a period of a few days, other cytokines such as IL-8 lead to a significant increase in the number of PBSC within hours (Fibbe et al., 1999).

As has been described with the heart following AMI, a stroke also triggers the release of cytokines that induce the mobilization of BMSC and their migration into the brain. For example, the number of PBSC in stroke patients nearly tripled within 7 days after the stroke (Hennemann et al., 2008; Paczkowska et al., 2005). In one study, the magnitude of stem cell release was actually correlated with the functional recovery of the patients (Dunac et al., 2007). Interestingly, the number of circulating stem cells did not increase in patients who received thrombolysis therapy immediately after their stroke. Therefore, it appears that it is

the lingering injury that slowly leads to the mobilization of stem cells from the bone marrow.

Finally, injuries to the skin and bones were also shown to trigger mobilization of BMSC and their migration into the injured tissue. For example, within 24 hours of a severe burn, a rapid increase of up to 9-fold in the number of PBSC has been observed in the blood of burn patients (Fox et al., 2008). Furthermore, the size of the area of the body affected by the burns strongly correlated with the magnitude of the mobilization. The affected skin also released cytokines such as SDF-1 and VEGF, which are involved in the migration of PBSC to the skin and their differentiation into blood vessels, respectively (Mansilla et al., 2006). In one study (Lee et al., 2008), the number of PBSC peaked around 3 days after a bone fracture and rapidly returned to basal level within a few days. These results were confirmed in another study in which stem cells were shown to migrate to the fracture site and to promote neovascularization. The formation of new vessels was shown to peak at the fracture site 7 days after the fracture, which corresponds to the early phase of ossification of the fracture line (Matsumoto et al., 2008). Therefore BMSC mobilization was documented to naturally follow an injury or even be associated with chronic conditions.

The natural process by which stem cells are mobilized from the bone marrow is still not fully understood. Contrary to most tissues in which SDF-1 is secreted consequent to an injury or a degenerative condition, in the bone marrow SDF-1 is constitutively produced and released, and binding of SDF-1 to its exclusive receptor CXCR4 leads to the externalization of adhesion molecules, namely integrins, which allow for the adherence of stem cells to the bone marrow matrix. The binding of SDF-1 to CXCR4 is referred to as the SDF-1/CXCR4 axis. The general understanding is that disruption of the SDF-1/CXCR4 axis reduces the expression of adhesion molecules, leading to a reduction in the adherence of stem cells to the bone marrow matrix and the consequent mobilization of stem cells. Various compounds known to trigger stem cell mobilization all affect the SDF-1/CXCR4 axis in various ways.

For example, G-CSF disrupts the SDF-1/CXCR4 axis by activating a series of proteolytic enzymes including elastase, cathepsin G, and various matrix metalloproteinases (MMP2 and MMP9) that inactivate SDF-1 (Mannello et al., 2006; Jin et al., 2006; Carion et al., 2003). AMD3100 is a newly developed BMSC mobilizer and it acts by blocking CXCR4, disrupting the SDF-1/CXCR4 axis (Broxmeyer et al., 2005). A blocker of L-selectin was recently isolated from the cyanophyta *Aphanizomenon flos-aquae* and shown to trigger BMSC mobilization (Jensen et al., 2007). Inhibition of L-selectin leads to a down-regulation of CXCR4 expression, partially disrupting the SDF-1/CXCR4 axis. The mobilization mechanism of IL-8, SCF, VEGF and Gro β are not well understood.

Therefore, the human body has a mechanism to naturally mobilize BMSC which can then traffic to various areas of the body and contribute to tissue regeneration and repair.

2.2 Extravasation & recruitment

Recruitment is the process by which PBSC are recruited by a specific tissue signaling for repair. The process of PBSC recruitment in a tissue takes place predominantly at the level of the postcapillary venule, in a manner similar to neutrophils (Henschler et al., 2008).

In brief, the sudden drop in blood pressure taking place at the postcapillary venule triggers turbulence whose shear force mechanically activates L-selectin which in turn triggers the externalization of CXCR4, making PBSC more responsive to signals coming from tissues. If the tissue is in need of repair, it is secreting SDF-1 as well as other compounds such as stem cell factor (SCF) and hepatocyte growth factor (HGH) (Kucia et al., 2004; Neuss et al., 2004) that diffuse locally to the capillaries. Binding of SDF-1 and SCF to their specific receptors (e.g. CXCR4 and c-Kit) leads to the expression of adhesion molecules at the surface of stem cells (Voermans et al., 2000; Peled et al., 1999; Peled et al., 2000). Through a complex interaction of microvilli at the surface of both the capillary and PBSC, the stem cells initiate the process of tethering and then arrest on the capillary endothelium (Middleton et al., 1997; 2002).

Following firm attachment, SDF-1 and HGH continue to bind to their respective receptors CXCR4 and c-met at the surface of SC, which triggers the release and activation of matrix metalloproteinases (MMPs) which digest the endothelial lining, allowing for the extravasation of PBSC (Mannello et al., 2006; Janowska-Wieczorek et al., 2000; Neuss et al., 2004; Ries et al., 2007).

When studying the migration behavior of SCs to a wide array of chemokines, SCs were found to migrate only toward SDF-1 (Wright et al., 2002; Jo et al., 2000). The migration to SDF-1 is a polarized phenomenon toward the chemokine source (chemotactic) and not a simple random motion (chemokinetic), as SCs only migrated in a gradient of SDF-1 and not when SDF-1 is uniformly distributed in the media.

SDF-1 is normally secreted, to some extent, by cardiomyocytes (Askari et al., 2003), skeletal muscles (Ratajczak et al., 2003), liver (Hatch et al., 2002; Kollet et al., 2003), brain (Bagri et al., 2002; Lazarini et al., 2003; Zou et al., 1998), and kidney (Schradler et al., 2002). However, its secretion increases during tissue damage such as AMI (Wojakowski et al., 2004; Abbott et al., 2004), ischemia (Takahashi et al., 1999; Iwaguro et al., 2002), toxic liver damage (Kollet et al., 2003; Swenson et al., 2008; Hatch et al., 2002), and excessive bleeding (Ratajczak et al., 2004).

In the tissue, the process of migration toward the site of injury relies on the interaction between CD44 and its ligand hyaluronic acid (HYA). HYA is one of a family of polysaccharides known as glycosaminoglycans (GAGS), typically found in the connective tissues of vertebrates. Alongside other proteins such as collagen, elastin, fibrillin, fibronectin and laminin, GAGS and HYA constitute the extracellular matrix (ECM) of most tissues. Studies in rats have shown that half of the HYA found in the body is in the skin, while 25% is found in joints and bones together (Reed et al., 1988). The rest is distributed somewhat equally in muscles and viscera (Clarris and Fraser, 1968; Comper and Laurent, 1978) where the highest concentrations are found in connective tissue that form the ECM of most tissues.

After extravasation, CXCR4 on the surface of SC and SDF-1 secreted by the effected tissue continue their interaction which, in the tissue, leads to the formation of pseudopodia (see Figure 1) toward the source of SDF-1 secretion. As SDF-1 binds to CXCR4 in the tissue, CD44 adhesion molecules are externalized at the tip of the pseudopodia, leading to adhesion to HYA pathways within the tissue. In the tissue, the binding of CD44 to HYA is transient, as CD44 molecules shed soon after binding to HYA (Friedl et al., 1995), thus enabling pseudopodia detachment from the ECM. CD44 can also be cleaved by specific enzymes whose secretion is enhanced by SDF-1 (Heissig et al., 2002; Okamoto et al., 1999;

Janowska-Wieczorek et al., 1999). Through this process, stem cells can migrate within the tissue toward the site of injury.

The fact that PBSC primarily migrate to organs affected by an injury or a degenerative process has been documented in several studies. For example, in the cases of male recipients of liver transplants from female donors, biopsies performed 4-13 months after transplantation contained a significant number of Y-chromosome positive hepatocytes (16% to 43%). In one patient who suffered from hepatitis C after liver transplant and died 4.5 months after transplantation, up to 43% of the transplanted liver was made of Y-chromosome positive hepatocytes in comparison with 5% after 4.5 months in a woman with a sound liver who received a bone marrow transplant from a man (Theise et al., 2000). Investigations carried out on archival autopsy and biopsy liver specimens obtained from two women who received a bone marrow transplant from male donors for the treatment of leukemia revealed that 5 to 10% of the liver had been replaced by donor derived BMSCs after 4.5 and 13 months, respectively (Theise et al., 2000).

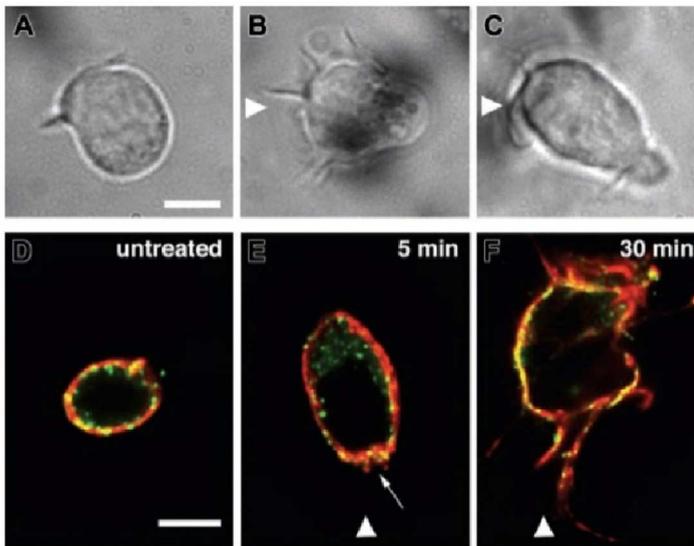


Fig. 1. CD44 is localized to the leading edge of polarized human stem cells migrating toward SDF-1. Cord blood-derived CD34⁺ cells were plated on HA coverslips and allowed to adhere for 30 minutes before recording cell movement. The position of SDF-1 source is indicated by arrowheads. (A-C) Phase contrast microscopy of untreated cells (A), cells stimulated with polarized source of SDF-1 (B), and cells treated with anti-CD44 mAb F10-44-2 and stimulated with polarized source of SDF-1 (C). (D-F) Cells treated as above were fixed 5 and 30 minutes after exposure to polarized source of SDF-1 and indirectly immunolabeled with antihuman CD44 mAb (red) and anti-CXCR4 mAb (green). An arrow is pointing to the fine CD44-positive protrusions at the direction of SDF-1. Bars = 5 μ m. (Taken from Avigdor et al., 2004)

Similar observations were made on men who received cardiac transplants from female donors. Analyses of tissue samples from biopsies revealed that an average of 0.1% (Muller et

al., 2002) to 15% of cardiomyocytes were Y-chromosome positive (Quaini et al., 2002; Laflamme et al., 2002). In one patient who died of cardiac rejection, 29% of the cardiomyocytes contained the Y chromosome in "hot spots" of high cardiac repair. In male patients who received lung transplants from female donors, recipient-derived BMSCs cells were detected as bronchial epithelial cells, type II pneumocytes, and seromucous glands in the transplanted lungs of all tested patients. In patients suffering from chronic damage, up to 24% of bronchial epithelial cells carried the Y-chromosome, indicating ongoing repair of damaged tissue by the recipient's own stem cells (Kleeberger et al., 2003).

In one study investigating this process of tissue repair by BMSC, irradiated mice were transplanted with GFP-positive SC before being injected in the right tibialis muscle with a large dose of cardiotoxin, which led to a loss of mobility within a few days. Yet, after eight weeks the injured muscle showed massive regeneration, with the right tibialis muscle significantly reconstructed with GFP-positive myocytes. By contrast, the contralateral non-injured leg showed very small incorporation of GFP-positive myocytes (Drapeau et al., 2010). Similar observations were made by Abedi et al. (2004), using smaller injections of cardiotoxin. Four weeks after the injection of cardiotoxin in the right leg, the area of the injection contained 1-2% of GFP-positive muscle cells while the left leg showed no fluorescence at all.

Similar observations were made in models of skin injury (Abedi et al., 2004). In a similar protocol, irradiated mice were transplanted with GFP-positive stem cells. BMSCs were then mobilized during five days using G-CSF. On the fourth day, mice were subjected to punch biopsies on their flank. The area of the injury was rebiopsied and sutured 48 hours and 1 month after the injury, in order to assess the incorporation of GFP-positive cells in the healing skin. While analysis at 48 hours showed significant infiltration by GFP-positive undifferentiated stem cells in the deep layer of the skin (hypodermis), after 4 weeks there was a large number of GFP-positive tissue cells in the dermis composing the structure of the healed skin, such as keratinocytes, sebaceous glands, blood vessels, and some rare muscle fibers and hair follicles. In the control animals that received G-CSF but no punch biopsy, none of these skin structures were positive for GFP, indicating that the few SC that had migrated in the skin had done so randomly.

In other studies looking at the incorporation of BMSCs in injured tissues, directed migration was demonstrated in the gut after section of an intestinal segment, (Hayakawa et al., 2003) in the heart after AMI (Orlic et al., 2001a; Fukuhara et al., 2004) or induced cardiomyopathy, (Hisashi et al., 2004) in the brain after stroke, (Sanchez-Ramos et al., 2002; Hoehn et al., 2002) and in the liver after drug-induced liver damage (Abedi et al., 2004). Taken altogether these studies clearly establish that BMSCs primarily migrate to areas subjected to injury, damage or simple degeneration.

2.3 Proliferation

When stem cells reach the site of an injury they must proliferate and expand, as there are not enough PBSC to accomplish full repair of any significant injury or degenerative process.

Several chemokines such as SDF-1 have been reported to enhance stem cell proliferation (Bonavia et al., 2003). The direct effect of SDF-1 on cell proliferation and survival is not well understood, but SDF-1 has been found to stimulate the proliferation and survival of stem

cells under certain experimental conditions (Broxmeyer et al., 2003). In tissues, SDF-1 appears to act as a “cellular survival factor” (Hwang et al., 2006). Insulin-like growth factor (IGF-1), when coupled with epidermal growth factor (EGF) or fibroblast growth factor-2 (FGF-2), was shown to support the proliferation and survival of neural (Arsenijevic et al., 2001) and muscle stem cells (Deasy et al., 2002). Extracellular nucleotides were also shown to support the proliferation of brain stem cell (Mishra et al., 2006).

2.4 Differentiation

The ability of adult stem cells to differentiate into various cell types has been well documented, though the mechanism behind such transformation is still not well understood.

As reported by Krause et al. (2001), 11 months after injection of male stem cells in female mice, Y-chromosome bearing cells were found in various tissues including the liver, muscle, skin, lung, and intestine. It has been well demonstrated that BMSC can differentiate into a wide variety of cell types including myocytes (Ferrari et al., 1998), hepatocytes (Lee et al., 2004), epithelial cells (Krause et al., 2001), neurons (Mezey et al., 2003; Sanchez-Rarnos et al., 2000; Woodbury et al., 2000), retinal cells (Tomita et al., 2002), endothelial cells and cardiomyocytes (Jackson et al., 2001; Orlic et al., 2001), gastrointestinal epithelium (Krause et al., 2001; Okamoto et al., 2002), pancreatic endocrine cells (Janus et al., 2003), bone and cartilage (Pereira et al., 1995; 1998).

Although little work has been done in this field and many questions remain to be answered, two possible mechanisms have been proposed for SC differentiation.

One proposed mechanism is cellular fusion, which takes place when two cells fuse together to become one cell. A few studies have suggested that SC have the ability to fuse with somatic cells, rescuing the target cell (Tarada et al., 2002; Vassilopoulos et al., 2003; Spees et al., 2003). Although there is clear evidence that this phenomenon did take place in a few experiments and that it may take place naturally in certain tissue such as the heart (Nygren et al., 2004), it is unlikely to be a significant physiological phenomenon (Wurmser and Gage, 2002). For example, while the process of fusion involves the interaction of one single SC with one somatic cell, therefore a ratio of 1:1, the extent of SC-mediated tissue repair that has been documented in numerous studies, involving various tissues, far exceeds the actual number of SCs migrating in the tissue. Furthermore, the process of cellular fusion results in a cell that is tetraploid. Although this has been observed in a few *in vitro* studies, it has been a very rare observation *in vivo*. In fact, even *in vitro*, relatively harsh conditions had to be used in order to obtain cellular fusion. Finally, cellular fusion would imply the merging of two different cellular membranes, a process that in itself is rigged with challenges, as cells are designed not to fuse.

So, although cellular fusion could possibly naturally take place in the body, it is unlikely to contribute significantly to the process of repair that has been documented with ASCs. The other most likely mechanism is differentiation through contact with cellular components when the affected tissue is locally subjected to the action of various matrix metalloproteinases (MMPs) or differentiation induced by cytokines released by neighboring cells.

This process was beautifully put in evidence by the study of Jang et al. (2004) where hematopoietic stem cells (HSC) were co-cultured with either normal or damaged liver tissue separated by a semi-permeable membrane with 0.4 μm pores. Using immunofluorescence to detect markers specific to either HSC (CD45) or hepatocytes (albumin), the authors could follow the transformation of the HSC population. When HSCs were cultured alone for 8 hours, they only expressed CD45 and no albumin. However, when HSCs were exposed to injured liver tissue, they rapidly became positive for albumin. Over time, the population of cells positive for CD45 began to decrease as the population positive for albumin began to increase. Albumin-positive cells were seen as early as 8 hours and constituted 3.0% of the cell population at 48 hours. The conversion was minimal and delayed when HSC were exposed to undamaged liver (control for injury). Therefore the presence of an injury appears to be an important factor in the process of SC differentiation into a specific type of somatic cells.

The authors further investigated the phenomenon of differentiation by tracking the presence of various markers found in developing fetal liver cells, such as αFP , and in mature hepatocytes, such as CK18, albumin, fibrinogen, and transferrin. They found that αFP was expressed only at 8 hours and was lost thereafter. On the other hand, the expression of CK18, albumin, fibrinogen and transferrin each increased over time. While at time 0 the HSCs expressed only CD45, after as little as 8 hours all liver-specific markers were positive. So during the differentiation process, the SC seemed to take a route similar to the development of hepatocytes in the developing fetus, leading to mature hepatocytes within less than 48 hours. This retracing of fetal development has also been documented in cardiopoiesis (Behfar et al., 2008).

In this study, differentiation did not involve cellular fusion, as the new liver cells only contained one set of chromosomes. The differentiation was necessarily triggered by signaling molecules produced by the damaged tissue. It has been suggested that MMPs produced by damaged tissue could be playing an important role in SC differentiation by digesting specific ECM components that would then diffuse and get into contact with SCs. Binding of such compounds to specific receptors would activate internal messengers that would trigger the process of differentiation by activating specific genes, as suggested by the high level of mRNA found in differentiating cells. (Mannello et al., 2006; Chavey et al., 2003)

Gap junction intercellular communication (GJIC) and tunneling nanotubes (TNTs) could constitute other mechanisms playing a significant role in SC differentiation, by direct cell-to-cell contact (Behfar et al., 2010). The diameter of one gap junction is around 2 nm and the molecular size cut-off level is around 1-2 kDa, which is sufficient for the intercellular exchange of ions, nucleotides and even small proteins (Neijssen et al., 2005). GJIC is known to play a crucial role in modulating several cellular functions, to the point that impaired or lack of GJIC has been associated with severe diseases (Dasgupta et al., 1999). It has been suggested that GJIC could play an important role in SC differentiation (Loewenstein and Rose, 1992). Likewise, recent studies indicated that two cells can also exchange information via TNTs (Rustom et al., 2004). TNTs would form a cytoplasmic bridge between two cells that could be large enough to allow the transport of large molecules or even whole cell organelles. Such information could play a role in the finalizing process of differentiation, though much work needs to be done in this field before we obtain a better understanding of the role of GJIC and TNTs in stem cell differentiation.

2.5 Paracrine effect

Finally, a number of studies investigating the effect of BMSC on various diagnostic entities have revealed that oftentimes the extent of the benefits observed cannot be accounted for by the sheer number of BMSC that have differentiated into somatic cells, even when there is clear evidence of tissue regeneration mediated by newly formed cells. For example, irradiated female mice transplanted with transgenic GFP+ BMSC showed much better recovery from experimental stroke after G-CSF-induced BMSC mobilization when compared to non-mobilized control mice (Kawada et al., 2006). Both motor and cognitive functions were improved by BMSC mobilization, and the treatment also reduced infarct size. However, using bromodeoxyuridine (BrdU), it was observed that a significant number of the newly formed brain cells did not derive from BMSC but rather from local neural stem cells. Similar results were seen with spinal cord lesion and muscle injury (Kinnaird et al., 2004; Osada et al., 2010). The regenerative effect is believed to be triggered by the secretion of growth factors and paracrine signaling by BMSC (Uccelli et al., 2011).

The paracrine effect has been best put in evidence in the heart after AMI. Delivery of BMSC to ischemic cardiac tissue has led to a significant increase in the concentration of IL-10, interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α) and other cytokines in the cardiac tissue, which contributed to neovascularization and reduction of infarct size (Kamihata et al., 2001; Burchfield et al., 2008; Gneccchi et al., 2005; Mirotsoiu et al., 2011). Condition media in which BMSC were exposed to hypoxia proved to be cytoprotective to cardiomyocytes and was able to reduce infarct size (Gneccchi et al., 2006).

In all, a growing body of evidence supports the hypothesis that as BMSC migrate into tissues, aside from differentiating into cells of the target tissue, they also exert their regenerative effect at least in part -and maybe even to a large extent- through the secretion of paracrine signaling compounds.

3. ESCM as a treatment approach

Given the fact that SDF-1 is secreted by various organs and tissues upon injury or degeneration, it would follow that the number of PBSCs should be an important parameter in the overall effectiveness of SC-mediated tissue repair and regeneration. A higher number of PBSC would mean more SC available to respond to SDF-1 signaling and migrate into tissues. In this regard, Tomoda et al. (2003) reported that after a heart attack individuals with a higher number of PBSC showed greater recovery of cardiac functions after 6 months when compared to people having fewer PBSC at the time of the cardiac event.

In a prospective study using more than 500 individuals, Werner et al. (2005, 2007) put in evidence that the number of PBSC is a critical parameter in the role of SCs in tissue repair. The authors quantified the baseline number of PBSC in 519 individuals (average 66.6 \pm 10.8 years old) at risk for cardiovascular problems, and monitored their condition for one year. A first major cardiovascular event occurred in 214 patients. After adjustment for age, sex, vascular risk factors, and other relevant variables, increased levels of PBSC were associated with reduced risk of death from cardiovascular causes, lesser risk of a first major cardiovascular event, greater revascularization and lesser frequency of hospitalization.

Similar data has been obtained by Vasa et al. (2001) who documented that a higher number of endothelial progenitor cells (EPC) was associated with greater cardiovascular health. In fact, hypoxia has been associated with the secretion of SDF-1 and VEGF by the ischemic tissue (Schioppa et al., 2003; Bachelder et al., 2002). Circulating EPCs therefore are attracted to the ischemic heart by the action of SDF-1 and the migrating EPCs contribute to the formation of new blood vessels upon the action of VEGF (Lee et al., 2007). Therefore, a higher number of EPCs or PBSC helps maintain optimal blood flow and a strong cardiac tissue.

The link between disease formation and the number of circulating stem cells is not limited to the heart. Similar observations have been made with muscular dystrophy where the rate of progression of the disease has been linked to the number of circulating stem cells and for which the number of PBSC is now considered one of the most important predictors of the disease progression (Marchesi et al., 2008). Likewise, the progression of pulmonary arterial hypertension (Diller et al., 2008; Junhui et al., 2008), arthritis (Herbrig et al., 2006; Grisar et al., 2005), atherosclerosis (Zhu et al., 2006), lupus erythematosus (Westerweel et al., 2007; Moonen et al., 2007), kidney failure (Choi et al., 2004; Herbrig et al., 2004; Eizawa et al., 2003), migraine (Lee et al., 2008), erectile dysfunction (Baumhake et al., 2007) and other diseases have all been linked to a reduction in the number of PBSC. Recently, a direct relationship has been established between the number of PBSC and the development of diabetes, linking impaired fasting glucose, impaired glucose tolerance, and insulin-dependent diabetes mellitus to progressively lower levels of PBSCs (Fadini et al., 2010).

If the number of PBSC constitutes such a key parameter in the process of SC-mediated tissue repair, therefore increasing the number of PBSC could constitute a therapeutic approach. Following is the description of a number of clinical trials investigating the clinical potential of ESCM in various diseases or injuries.

3.1 Acute myocardial infarction

The heart has been traditionally seen as having little regenerative capabilities after birth, although many recent studies have challenged this view. Evidence clearly suggests that there is a low level of constant regeneration of cardiac cells (Soonpaa and Field, 1998; Bergmann et al., 2009; Quaini et al., 2002), and the number of dividing cells can increase by up to 10-fold in chronic heart disease or after AMI (Kajstura et al., 1998; Beltrami et al., 2001). Yet, this level of proliferation seems insufficient to rescue the cardiac muscle after AMI (Schwartz and Kornowski, 2003) and survivors of heart attacks are left with reduced quality of life and little prospect for improvement.

A number of studies in animals using G-CSF and SCF have shown that ESCM can lead to significant cardiac repair after AMI. Injection of SCF and G-CSF for 8 days after inducing AMI significantly increased the number of PBSCs, which led to the migration of PBSCs into the myocardium (Orlic et al., 2001b). Twenty-seven days after AMI, a band of newly formed cardiac tissue occupied more than 75% of the infarcted region of the ventricle and newly formed blood vessels were supplying the infarcted tissue. The blood vessels were surrounded by smooth muscles and microscopic observations revealed the presence of red blood cells, indicating that the newly formed arterioles integrated structurally with the remaining functional vasculature. By comparison, in control animals the ventricular wall

was filled with scar tissue covering the entire area of the infarct and no new blood vessels could be seen.

In summary, while only 17% (9 of 52) of the untreated animals survived AMI, showing severe signs of cardiomyopathy and compromised blood circulation, up to 73% (11 of 15) of the animals treated with G-CSF survived with significantly improved cardiac function and restored blood circulation. After 27 days, ejection fraction was 114% greater in the treated group and other parameters such as end-diastolic pressure, systolic pressure, and other parameters of cardiovascular function were all improved in treated versus non-treated mice.

Injection of G-CSF, however, can have significant negative effect in humans if done at large dose for more than 5-6 days (Bensinger et al., 1996; Shimoda et al., 1993). At lower dose and with shorter treatment duration, human trials have so far delivered mitigated results, though the approach remains promising. While some groups did report very promising results (Ince et al., 2005; Sesti et al., 2005), others reported no effect at all (Ellis et al., 2006; Zohnhofer et al., 2007; Ripa and Kastrup, 2008). A comprehensive review of the various studies however reveals that each study used slightly different protocols with regard to the time of treatment after AMI (from hours to 3 months), as well as the intensity and duration of the treatment, suggesting that ESCM could indeed hold great promise once the most effective treatment protocol has been developed (Abdel-Latif et al., 2008).

For example, Wojakowski et al. (2006) reported in 43 cardiac patients that if the patients were treated early after AMI (<12 hours) with G-CSF, the number of PBSCs following BMSC mobilization correlated with the extent of cardiac repair. In another study, G-CSF was injected within 5 days post-AMI in 41 patients at high risk for unfavorable left ventricular remodeling. Five months after G-CSF treatment, ejection fraction had improved 12.5% compared to no improvement in the control group (Leone et al., 2007). The improvements in cardiac function appeared to be linked to the prevention of left ventricular remodeling.

A meta-analysis reviewing the effectiveness of BMSC mobilization for the treatment of AMI included 7 studies and a total of 364 patients. The analysis concluded that treatment with G-CSF can improve LV ejection fraction if the treatment is administered early after the heart attack (Kang et al., 2007). However, in spite of the improvements in ejection fraction, other general parameters of cardiovascular health such as ventricular arrhythmia, rehospitalization for heart failure, and the composite of other cardiovascular events (i.e., death from heart attack, recurrent heart attack, and stroke), were not significantly different in the G-CSF treatment groups compared with the control groups. Similar results were reported by another meta-analysis that included eight studies and 385 patients (Abdel-Latif et al., 2008).

So, it remains unclear whether the simple mobilization of BMSCs can constitute an effective treatment for AMI. While some studies have yielded promising results, others suggest no benefits at all. However, positive results obtained in some studies should not be denied on the basis of the negative results obtained in others. Reconciliation of all this data and the development of an effective treatment protocol will most probably come through the determination of optimal treatment parameters: 1) intensity of ESCM, 2) duration of the treatment, 3) time after AMI, 4) number of treatments received over time, and 5) other yet unidentified parameters. Compounds other than G-CSF might also be discovered that could provide more consistent results (Broxmeyer et al., 2005; DeClercq, 2005).

3.2 Stroke

Many studies have shown that extensive neuronal death in the brain after a stroke triggers the migration of neural stem cells to the site of injury, followed by their proliferation and differentiation into neurons and glial cells (Peterson, 2002; Fallon et al., 2000; Arvidsson et al., 2002; Nakatomi et al., 2002; Schmidt and Reymann 2002). However, this natural process does not appear to be sufficient to produce significant functional recovery (Yamamoto et al., 2001; Magavi and Macklis, 2002).

As with the heart after AMI, stroke has been associated with BMSC mobilization. Studies have shown that the number of PBSC in stroke patients can increase up to 3-fold within 7 days after the stroke (Hennemann et al., 2008; Paczkowska et al., 2005). In one study, the magnitude of BMSC mobilization was correlated with the patients' functional recovery (Dunac et al, 2007).

When rats were injected with rat (Chen et al., 2001; Pavlichenko et al., 2008; Willing et al., 2003) or human SC (Li et al. 2002) after an induced stroke, significant motor and cognitive improvements were observed. Although a significant number of BM-derived cells could be identified as newly formed neurons and glial cells in the stroke foci, they accounted for only a small percentage of the total number of newly formed brain cells. Most of the newly formed brain cells are believed to be derived from neural SC upon the action of paracrine factors secreted by the migrating SC. Similar results were obtained using human umbilical cord stem cells (HUCSCs) where intravenous injection of HUCSCs 24 hours after a stroke greatly improved functional recovery (Chen et al., 2001). Injection of HUCSCs 7 days after the stroke still led to significant functional recovery, though the extent of the recovery was less than with treatment at 24 hours.

Mobilization of BMSCs induced by G-CSF was shown in a number of studies to improve the outcome of a stroke. For example, when tested 14 and 28 days after a stroke, animals treated with G-CSF showed much greater body coordination than control animals (Shyu et al., 2004). When the brains were analyzed using imaging, the infarcted area was much smaller in the treated group (61 mm³) when compared to the control group (176 mm³). All these benefits were greatly reduced when the animals were pre-treated with a blocker of CXCR4, indicating that the observed effects were dependent upon the migration of BMSCs into the brain.

Similar results have been reported by other scientific teams (Six et al., 2003; Kawada et al., 2006). For example, after repopulating the bone marrow with GFP-positive stem cells, G-CSF-induced BMSCs mobilization immediately and roughly 2 weeks after inducing a stroke dramatically improved motor and cognitive performances four weeks after the stroke, as measured by using the Morris water maze (Kawada et al., 2006). In this study, while all the mice in the treated group reached the platform within 40 seconds, none of the control mice reached the submerged platform within the allotted 120 seconds. As in the study by Shyu et al. (2004), the infarct size was much smaller in the treated animals when compared to control. Using BrdU it was observed that the number of new brain cells found in the infarcted area was much higher in the treated group than in the control group. Yet very few of the new brain cells were GFP-positive, supporting the view that as they migrate in the brain BMSCs secrete growth factors that support the proliferation and differentiation of neural stem cells (Yoo et al., 2008). BMSCs also support neovascularization, which further

contributes to the regeneration of the brain tissue (Lee et al., 2005; Hess et al., 2002; Kan et al., 2005).

Although much of this work needs to be reproduced in humans, ESCM for the treatment of stroke appears promising and would constitute a safe approach to the treatment of stroke.

3.3 Diabetes

The ability of BMSCs to leave the bone marrow, migrate to the pancreas and become insulin-producing cells was beautifully shown by Ianus et al. (2003). In brief, female mice were lethally irradiated and then transplanted with male BMSCs that express, using a CRE-LoxP system, GFP if the insulin gene is actively transcribed. When analyzed 4-6 weeks after the transplantation, GFP-positive cells were found in the pancreas (Figure 2). The GFP-positive

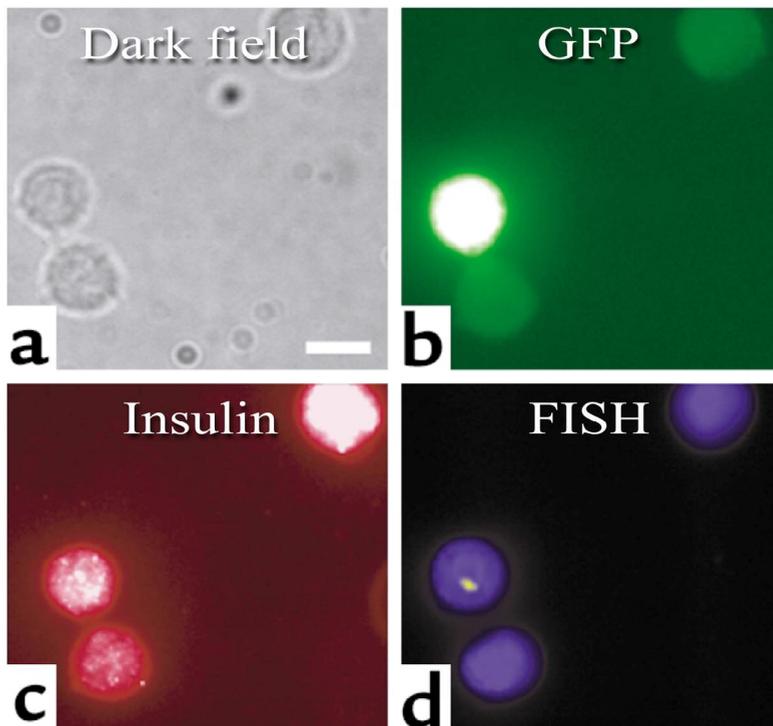


Fig. 2. FISH and immunofluorescence marking of BM-derived insulin-producing cells. Immunofluorescence and FISH of isolated, dispersed pancreatic islet cells after transplantation of lethally irradiated female mice with male BMSCs that express, using a CRE-LoxP system, GFP if the insulin gene is actively transcribed. a) Bright-field phase, b) GFP imaging note slight autofluorescence of control isolated islet cells; c) Immunostaining with rhodamine X-labeled secondary antibody for insulin; d) FISH for Y chromosome (in yellow) and nucleus stain with DAPI (blue). Y chromosome is present only in GFP-positive cells. Scale bar, 5 μ m; X630. (Taken from Ianus et al., 2003)

cells were also positive for insulin, for insulin RNA, and for Y-chromosome, demonstrating that they originated from the transplanted BMSCs. These cells showed functional characteristics typical of normal pancreatic β -cells, such as fluctuations of intracellular calcium upon exposure to various concentrations of glucose. Within the time frame of that study (4–6 weeks), 1.7–3% of BM-derived GFP-positive cells were detected in the pancreatic islets. In a similar study, BMSCs were also shown to participate into the development of new blood vessels, further supporting the regeneration of the pancreatic tissue (Mathews et al., 2004; Gao et al., 2008).

Then, using a protocol similar to that used by Ianus et al., Hasegawa et al. (2007) further demonstrated that mobilization of BMSCs was not only effective but essential for pancreatic regeneration. Hasegawa et al. induced diabetes by injection of streptozotocin (STZ) in lethally irradiated female mice followed by infusion of BMSC from GFP transgenic mice. Infusion of BMSCs led to the incorporation of GFP-positive BMSCs into islets of Langerhans in the pancreatic tissue, partially restoring pancreatic islet number and size, and improving STZ-induced hyperglycemia. However, when the same experiment was done while simply infusing the pancreas with BMSCs, without preirradiation, no improvement was obtained. Furthermore, when the experiment was repeated with full BMSC transplant in a model of mice with impaired ability to mobilize stem cells, no benefits were obtained. Therefore, natural mobilization of BMSCs from the bone marrow appears essential for the regeneration of pancreatic function after inducing diabetes with STZ.

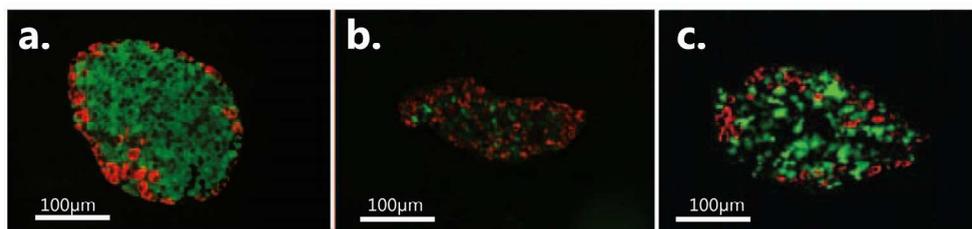


Fig. 3. Pancreatic islets of STZ-treated mice receiving subsequent bone marrow transplant (BMT). Double immunostaining of pancreases with anti-insulin and anti-glucagon antibodies. *Green* indicates insulin-positive and *red* glucagon-positive cells. Pancreases from normoglycemic control mouse (a), hyperglycemic control mouse (b), and STZ-treated mouse receiving BMT (c). BMT improved STZ-induced hyperglycemia. (Taken from Hasegawa et al., 2007)

In one recent study in humans, ESCM showed great promise in the treatment of diabetes. The study selected individuals recently diagnosed for diabetes and the treatment consisted of both stem cell mobilization and autologous stem cell transplant. The patients first received injections of G-CSF in order to harvest PBSC, followed later by autologous stem cell transplant and, 5 days post-transplant, a second round of G-CSF treatment. The endpoints monitored in the study were overall morbidity along with temporal changes in exogenous insulin requirements. Before the treatment, all patient required daily insulin injection. By the end of the study, 14 of the 15 patients had experienced insulin-free episodes ranging between 1 and 35 months (mean 16.2 months) (VOLTARELLI et al., 2007).

In this study the patients benefited from two instances of ESCM and one instance of autologous SC transplant. It is not possible to determine what were the respective contributions of the ESCM and SC injection, however it is likely that the mobilizations by themselves significantly contributed to the benefits experienced. While the first mobilization lasted several days and the second mobilization lasted about one week, there was only one instance of stem cell injection.

Diabetes is an interesting disease to study the link between disease progression and the number of circulating PBSCs, as it follows a series of relatively well defined stages with regard to carbohydrate metabolism status, namely normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and newly diagnosed diabetes mellitus (DM). Fadini et al. (2010) quantified the number of circulating CD34+ cells by flow cytometry in 425 individuals divided among these four stages of disease progression. The data showed a clear trend of decreased number of PBSCs with disease progression through IFG, IGT and DM (Figure 4). The number of circulating PBSCs was significantly lower in the IGT and DM groups when compared to the NGT group. The reduction in the number of PBSCs can either be a consequence of higher blood glucose levels that might affect the ability of stem cells to mobilize from the bone marrow or a causal factor in the development of DM whereby a reduced number of circulating PBSCs reduces the ability of the pancreas to renew itself over the years, or both. This supports the view previously suggested that diabetes could be a stem cell disease (Fadini et al., 2009).

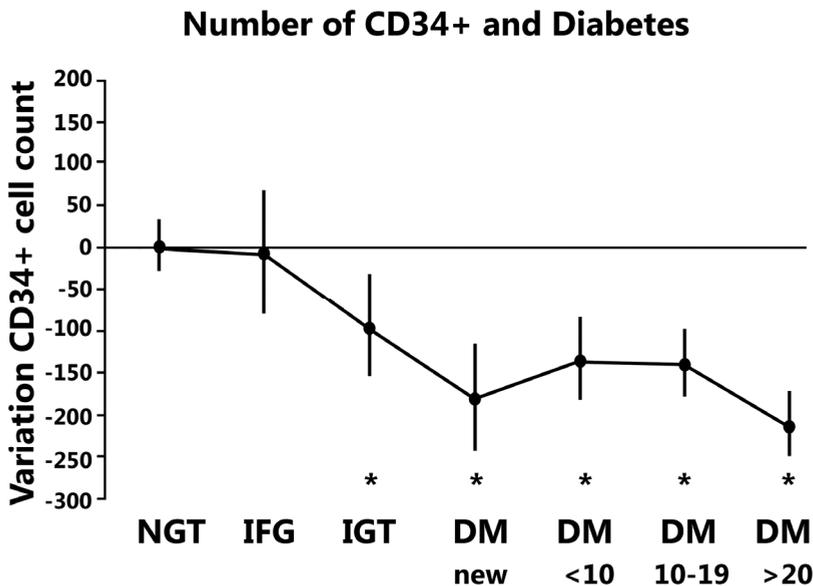


Fig. 4. Variation of circulating CD34+ cells and diabetes. Variation of circulating CD34+ cells in patients grouped according to carbohydrate metabolism, namely normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or diabetes (DM) duration, as appropriate. The mean value of patients with NGT was taken to represent the zero point. Bars indicate 95% CIs of means. * Values significantly different when compared to NGT. (Taken from Fadini et al., 2009)

4. Clinical application (SE)

Investigation of the clinical potential of ESCM has been limited, largely due to the significant risk associated with the use of G-CSF, the main stem cell mobilizer used in clinical trial, for extended periods of time (Bensinger et al., 1996; Shimoda et al., 1993). Recently, a new stem cell mobilizer (StemEnhance®; SE) has been developed that triggers a much milder increase in the number of PBSC, but its safety allows for a sustained oral daily consumption over long periods of time, allowing for safe daily ESCM (Jensen et al., 2007).

In brief, SE is an extract from the cyanophyta *Aphanizomenon flos-aquae* that concentrates a protein with an estimated molecular weight of 160–180 kDa, which was shown to be a selective L-selectin blocker. Oral consumption of 1 gram of SE was shown to trigger an average 25% increase in the number of PBSC within 60 minutes after consumption. The magnitude of the mobilization induced by SE is much smaller than that triggered by G-CSF, however its safety allows for continuous use and therefore offers a novel approach in the study of ESCM. To test its therapeutic potential, SE was used in a number of preliminary clinical trials involving a number of diagnostic entities.

4.1 Parkinson

MC is a 62 year old male with a 17 year history of Parkinson's Disease. MC was initially diagnosed with Parkinson's type resting pill-rolling tremor affecting his left hand and then progressing to the limbs on left side of his body. Over the course of five years the tremors increased gradually affecting the right side as well. At ten years into the disease process, MC was no longer able to practice as an attorney, mobility was affected and limitations increased compromising his personal and professional abilities.

In 2009, before the treatment of SE, the left side tremors had significantly worsened, symptoms of stiffness and bradykinesia which introduced a shuffling gait and poor balance made it unsafe for MC to ambulate without the use of a cane. At the time MC began consuming SE he was unable to dress himself, put on his watch, write, feed himself or drive his car. After 45 days on the product, consuming 1 gram of SE, three times per day, MC showed significant improvement with decreased tremors, less stiffening and bradykinesia; at this time MC was able to get around without the use of his cane. After 60 days on the product, judging that the benefits had plateaued, MC discontinued the use of SE. Forty - five days later, signs of tremor and bradykinesia returned and MC was seeking medical attention, once again. MC resumed SE with a dose of 2 grams, three times per day and within six weeks showed much improvement with less tremors, stiffness and bradykinesia. MC was able to participate in activities of daily living, such as dressing and feeding himself, he was also able to tie his own tie, put on his watch, and once again, he could ambulate without using a cane. To date MC has been on SE for two years, he has returned to driving his car without limitations, he is independent with all activities of daily living and he also participates in some level of professional activity.

Another patient, MT, is a 52 year old woman with an early onset of Parkinson at age 36, with tremor as the primary symptom. Early treatment consisted of Pergolid, then Budipin up to 30 mg three times a day. However due to QTc increase, Budipin was later reduced to 10 mg three times a day. The patient was also treated with Methyldopamin 62.5 mg four times a day. With this treatment, MT's main problem was the experience of fluctuations and

the beginning of ON-OFF syndrome. One year ago MT began consuming StemEnhance 1 gram three times a day. Today MT's experiences only minor tremor accompanied with some dyskinetic syndrome and sometimes propulsion. Her quality of life has increased and she is much more socially active.

4.2 Traumatic spinal cord injury

A preliminary trial with 8 individuals with spinal cord injuries was performed in a community center in Hawaii. The cases all involved paraplegia and various degrees of quadriplegia. Of the 8 cases, 4 dropped due to various circumstances unrelated to the consumption of SE. Of the 4 remaining participants, 2 had repetitive periods of hospitalization that made their consumption of SE irregular. Of the two remaining participants, one experienced mild though significant improvement in mobility while the other participant experienced significant improvements in mobility.

The latter participant, VS, had a serious car accident 17 years prior to SE consumption and was left with traumatic brain injury that affected her speech and a significant spinal cord lesion. At the beginning of the trial, VS was able to lift her right leg approximately 15 centimeter from her chair with no lateral movement, and showed a total absence of movement in her left leg. She could move her arms, hands and fingers though the movements were very slow with little dexterity and precision. She had some ability to move in her bed but was unable to turn herself without assistance. No data was available to document peripheral sensory perception of the lower limbs or nerve conductivity. After 6 months of daily consumption of 3 grams of SE three times a day, VS could lift her right leg more than 30 centimeters from her chair, with lateral movement outside of her chair. She could also lift her left leg off the chair and laterally outside of the chair. VS had less control over the movement of her left leg, though the magnitude of the movements was comparable to the movements seen with the right leg. After 10 months, VS was able to rotate herself in her bed unassisted and from a supine position she could lift both legs to a 90 degree angle and sit in her bed unassisted. Over the period of the trial her upper limbs also improved in dexterity and her speech showed mild though significant improvement. VS comes from a disadvantage socio-economic environment and did not have access to physical therapy beyond the first few years of her injury, she therefore developed leg and feet deformities that prevented her from bearing weight and possibly resuming physical therapy.

4.3 Coronary Artery Disease

JP is a 60 year old South African male who experienced a heart attack at 51 years of age. After diagnosis of Coronary Artery Disease was made a stent placement was performed. Dietary and lifestyle changes were implemented immediately by JP following the hospitalization. Unfortunately, 3 years later, JP suffered four additional heart attacks. During the hospitalization, the angiogram revealed the right and left coronary arteries were obstructed 100% and 40%, respectively, thus determining that JP was not a good candidate for bypass surgery. At the time of the last hospitalization, JP had decreased energy and experienced "stable" angina with any exertion, and his overall quality of life was greatly compromised. JP was put on a medication regimen which consisted of Atenolol 50mg daily,

Perindopril 4 mg daily, Elantan 20 mg twice per day, Simvastatin 20 mg daily, Adalat xl 30 mg daily, and Aspirin 1 per day. JP was then put on a list for possible heart transplant.

Three weeks after discharge JP began consuming SE, 1 gram three times per day. After 3 months of taking the product, he received a call for a possible heart transplant and returned to see his cardiologist for evaluation and comprehensive testing. The cardiologist reported that JP was making a remarkable recovery, the heart transplant surgery was postponed. Four months later JP was re-evaluated and found to have made a complete recovery. JP has since been returning at 6 month intervals for follow up evaluation, and to date he has been stable with no further coronary incidents. The evaluations and most recent ECG conclude that JP has normal heart function. Presently, JP shares that he is not on any medications, blood pressure is 126/65 mm Hg, he continues to take SE and experiences good quality of life.

4.4 Diabetes and rheumatoid arthritis

NA is a 47 year old Colombian woman who was diagnosed at 18 year of age with deforming rheumatoid arthritis. Over the years she managed her condition with the use of Prednisone, Methotrexate, Sulfasalazina, and Diclofenac. Four years ago NA was diagnosed with diabetes mellitus with glycemia reaching 308 mg/dL and assumed treatment with Euglucon 5 mg twice a day along with NPH insulin. About one year ago, due to her arthritic condition she became wheelchair bound and required assistance for bathing and dressing. At that time her medical records show levels of C-Reactive Protein (CRP) of 96.2 mg/L, Erythrocytes sedimentation rate (ESR) of 81 mm/H, Platelet count of 535 K/ul, fasting glucose of 147 mg/dL and HgbA1c (or Glycosylated Hemoglobin) of 8.07. A few months after becoming wheelchair bound NA began consuming 1 gram of SE once a day. After one month she subjectively reported a reduction in pain and inflammation, while 3 months later her mobility had improved to the point that she could bathe and dress alone. After 6 months she began using a walker, then switched to a cane and one year later she was walking unaided. Her last medical records indicate a level of CRP of 2.1 mg/L, ESR of 34 mm/H, Platelet count of 485 K/ul. NA has also discontinued the use of any diabetes medication; her records indicate a fasting glucose of 106 mg/dL with a glycemia not exceeding 120 mg/dL and HgbA1c of 5.62.

At the time of writing this report, NA does not take any anti-inflammatory drug, undergoes a remarkable improvement in her quality of life, keeping daily consumption for 15 consecutive months of 1 gram of SE, bone support supplementation of 500 mg Calcitriol (1,25-dihydroxycholecalciferol) every month, milk of magnesia and an annual dose of Zoledronic Acid.

4.5 Cerebrovascular accident (Stroke)

In September 2008, GE, a 78 year old male surgical oncologist who was otherwise in good health, had a stroke. The MRI/MRA revealed an acute infarct involving the right lentiform nucleus, moderately extensive chronic small vessel ischemic changes, chronic lacunar infarct involving the right ventromedial thalamus, and intracranial atherosclerotic vascular disease. The stroke left GE with aphasia and a reduced ability to perform any physical activity. October 2009, 13 months after the stroke, GE began taking SE; GE consumed 1 gram twice

per day and after 8 weeks on the product GE noticed improvement with his speech and experienced more energy with improved balance. In April 2010 a repeat MRI/MRA showed no evidence of an acute infarct. GE's aphasia was completely resolved at this point and his overall mobility was improved allowing him to perform all activities of daily living. In January of 2011 a repeat MRI/MRA of the anterior and posterior cerebral arteries demonstrated no evidence of hemodynamically significant stenosis, and revealed normal vertebrobasilar arteries with no evidence of intracranial aneurysm or vascular malformation.

To date, GE has resumed playing tennis at age 81, walks around the mall in the neighborhood with other senior citizens and has returned to his professional activity as a surgeon.

4.6 Kidney failure

The whole paragraph should read: "GW is 36 months old; he was born with a malfunctioning valve in the urethra, compromising the flow of urine at birth. At ten days old he had surgery to repair the urethra, however, the damage to the kidneys and the bladder were already evident, caused from the retention of urine at birth. Following the surgical procedure, scar tissue was observed to the wall of the bladder and one of the kidneys did not respond, eventually that kidney quit working and began to atrophy. A scar was also left on his hand caused from an IV being ripped out after surgery. Before SE was introduced, GW had stopped growing at 15 months of age and multiple health issues were leading him toward a kidney transplant. Furthermore, GW had been on antibiotics for one year due to multiple infections. His body was not eliminating fluid as it should, as evidenced by the worsening of edema resulting in the use of diuretics. GW had purple feet caused from compromised circulation, dark spots were observed on his back, his eyes were blood shot and his overall presentation was pale in appearance. GW's activity was nothing normal for a 1 ½ year old child; his energy level was very depleted and he was unable to play like a normal toddler.

GW was 21 months old when SE was introduced at 250 mg per day. After 3 days on SE his mother reported that his eyes were crystal clear and he was observed running through the house full of energy. The dark spots on his back began to fade after one week of SE consumption. Red color was seen on GW cheeks for the first time since he was born. After 75 days on SE, GW had grown 6cm taller and the shrinking kidney at birth had measured 0.8cm larger. GW is off all pharmaceutical medications to date and his bladder and kidney are back to normal functioning to their fullest capacity, the scar to his hand is completely gone.

5. Conclusion

The benefits of ESCM on various degenerative conditions have been documented in several animal models and in humans. In some cases, BMSC clearly migrate into tissues and directly contribute to the formation of new functional somatic cells of the target tissue. However in other cases, especially diseases affecting the heart and central nervous system, a primary mechanism of action appears to be the secretion of paracrines that stimulate the

proliferation and differentiation of tissue stem cells. Much work remains to be done to clearly elucidate the mechanisms of action behind the benefits of ESCM in various diagnostic entities, however from a clinical standpoint it remains that regardless of the mechanism of action, ESCM appears to be a valuable approach to increase the quality of life of patients affected by various degenerative diseases.

Various stem cell mobilizers have been documented in the scientific literature and many of them have been associated with side effects that prevent the application in humans of what has been documented as effective in various animal models. Such mobilizers include G-CSF, Stem Cell Factor, interleukin-8 and plerixafor (Lemery et al., 2011), which have all been associated with side effects going from diarrhea, nausea, pain and numbness to pericarditis and thrombosis. In spite of the potential benefits, such side effects have largely prevented the use of such compounds for ESCM in humans, and the lack of safe stem cell mobilizers largely explains the limited interest so far in this therapeutic approach. The main challenge in further investigating the therapeutic potential of ESCM remains therefore the development of safe stem cell mobilizers. In the meanwhile, SE appears to be a valuable tool to study the clinical benefits of ESCM.

6. References

- Abbott JD, Huang Y, Liu D, Hickey R, Krause DS, Giordano FJ. (2004) Stromal cell-derived factor-1 α plays a critical role in stem cell recruitment to the heart after myocardial infarction but is not sufficient to induce homing in the absence of injury. *Circulation*. 110(21):3300-5.
- Abdel-Latif A, Bolli R, Zuba-Surma EK, Tleyjeh IM, Hornung CA, Dawn B. (2008) Granulocyte colony-stimulating factor therapy for cardiac repair after acute myocardial infarction: a systematic review and meta-analysis of randomized controlled trials. *Am Heart J*. 2008 Aug;156(2):216-226.e9.
- Abedi M, Greer DA, Colvin GA, Demers DA, Dooner MS, Harpel JA, Pimentel J, Menon MK and Quesenberry PJ (2004) Tissue injury in marrow transdifferentiation *Blood Cells Mol Dis* 32(1):42 - 46
- Arufe MC, De la Fuente A, Fuentes-Boquete I, De Toro FJ, Blanco FJ. (2009) Differentiation of synovial CD-105(+) human mesenchymal stem cells into chondrocyte-like cells through spheroid formation. *J Cell Biochem*. 108(1):145-55.
- Arsenijevic Y, Weiss S, Schneider B, Aebischer P. (2001) Insulin-like growth factor-I is necessary for neural stem cell proliferation and demonstrates distinct actions of epidermal growth factor and fibroblast growth factor-2. *J Neurosci*. 21(18):7194-202.
- Arvidsson A, T Collin, D Kirik, Z Kokaia and O Lindvall. (2002). Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nature Med* 8:963-970.
- Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, Rovner A, Ellis SG, Thomas JD, DiCorleto PE, Topol EJ, Penn MS. (2003) Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. *Lancet*. 362(9385):697-703.
- Avigdor A, Goichberg P, Shivtiel S, Dar A, Peled A, Samira S, Kollet O, Hershkoviz R, Alon R, Hardan I, Ben-Hur H, Naor D, Nagler A, Lapidot T. (2004) CD44 and hyaluronic

- acid cooperate with SDF-1 in the trafficking of human CD34+ stem/progenitor cells to bone marrow. *Blood* 103(8):2981-9.
- Bachelder RE, Wendt MA, Mercurio AM. (2002) Vascular endothelial growth factor promotes breast carcinoma invasion in an autocrine manner by regulating the chemokine receptor CXCR4. *Cancer Res* 62:7203–7206.
- Bagri A, Gurney T, He X, Zou YR, Littman DR, Tessier-Lavigne M, Pleasure SJ. (2002) The chemokine SDF-1 regulates migration of dentate granule cells. *Development* 129:4249–4260.
- Baumhäkel M, Werner N, Böhm M, Nickenig G. (2006) Circulating endothelial progenitor cells correlate with erectile function in patients with coronary heart disease. *Eur Heart J*. 27(18):2184-8.
- Behfar A, Faustino RS, Arrell DK, Dzeja PP, Perez-Terzic C, Terzic A. (2008) Guided stem cell cardiopoiesis: discovery and translation. *J Mol Cell Cardiol*. 45(4):523-9. Epub 2008 Sep 26.
- Behfar A, Yamada S, Crespo-Diaz R, Nesbitt JJ, Rowe LA, Perez-Terzic C, Gaussin V, Homsy C, Bartunek J, Terzic A. (2010) Guided cardiopoiesis enhances therapeutic benefit of bone marrow human mesenchymal stem cells in chronic myocardial infarction. *J Am Coll Cardiol*. 56(9):721-34.
- Beltrami AP, Urbanek K, Kajstura J, Yan SM, Finato N, Bussani R, Nadal-Ginard B, Silvestri F, Leri A, Beltrami CA, Anversa P. (2001) Evidence that human cardiac myocytes divide after myocardial infarction. *N Engl J Med* 344:1750–1757.
- Bensinger W, Buckner W, Rowley S, Storb R, Appelbaum F. (1996) Treatment of normal donors with recombinant growth factors for transplantation of allogeneic blood stem cells. *Bone Marrow Transplant*. 17:S19-21.
- Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabé-Heider F, Walsh S, Zupicich J, Alkass K, Buchholz BA, Druid H, Jovinge S, and Frisén J. (2009) Evidence for cardiomyocyte renewal in humans. *Science* 324: 98-102.
- Bonavia R, Bajetto A, Barbero S, Porcile C, Pirani P, Ravetti JL, Zona GL, Spaziante R, Florio T, Schettini G. (2003) Chemokines and their receptors in the CNS: expression of CXCL12/SDF-1 and CXCR4 and their role in astrocyte proliferation. *Toxicol Lett* 139:181–189.
- Broxmeyer HE, Kohli L, Kim CH, Lee Y, Mantel C, Cooper S, Hangoc G, Shaheen M, Li X, Clapp DW. (2003) Stromal cell-derived factor-1/CXCL12 directly enhances survival/antiapoptosis of myeloid progenitor cell through CXCR4 and G proteins and enhances engraftment of competitive, repopulating stem cells. *J Leukoc Biol* 73:630–638.
- Broxmeyer HE, Orschell CM, Clapp DW, Hangoc G, Cooper S, Plett PA, Liles WC, Li X, Graham-Evans B, Campbell TB, Calandra G, Bridger G, Dale DC, Srouf EF. (2005) Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J Exp Med*. 201(8):1307-18.
- Burchfield JS, Iwasaki M, Koyanagi M, Urbich C, Rosenthal N, Zeiher AM, Dimmeler S. (2008) Interleukin-10 from transplanted bone marrow mononuclear cells contributes to cardiac protection after myocardial infarction. *Circ Res* 103(2):203-11.

- Can A, Balci D. (2011) Isolation, Culture, and Characterization of Human Umbilical Cord Stroma-derived Mesenchymal Stem Cells. *Methods Mol Biol.* 698:51-62.
- Carion A, Benboubker L, Hérault O, Roingard F, Degenne M, Senecal D, Desbois I, Colombat P, Charbord P, Binet C, Domenech J. (2003) Stromal-derived factor 1 and matrix metalloproteinase 9 levels in bone marrow and peripheral blood of patients mobilized by granulocyte colony-stimulating factor and chemotherapy. Relationship with mobilizing capacity of haematopoietic progenitor cells. *Br J Haematol.* 122(6):918-26.
- Chandra V, G S, Phadnis S, Nair PD, Bhonde RR. (2009) Generation of pancreatic hormone-expressing islet-like cell aggregates from murine adipose tissue-derived stem cells. *Stem Cells.* 27(8):1941-53.
- Chavey C, Mari B, Monthouel MN, Bonnafous S, Anglard P, Van Obberghen E, Tartare-Deckert S. (2003) Matrix metalloproteinases are differently expressed in adipose tissue during obesity and modulate adipocyte differentiation. *J Biol Chem* 278:11888 -11896.
- Chen J, Zhang ZG, Li Y, Wang L, Xu YX, Gautam SC, Lu M, Zhu Z, Chopp M. (2003) Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. *Circ Res.* 92(6):692-9.
- Chen X, Fang H, Schwob JE. (2004) Multipotency of purified, transplanted globose basal cells in olfactory epithelium. *J Comp Neurol.* 469(4):457-74.
- Choi JH, Kim KL, Huh W, Kim B, Byun J, Suh W, Sung J, Jeon ES, Oh HY, Kim DK. (2004) Decreased number and impaired angiogenic function of endothelial progenitor cells in patients with chronic renal failure. *Arterioscler Thromb Vasc Biol.* 24(7):1246-52.
- Clarris BJ, Fraser JR. (1968) On the pericellular zone of some mammalian cells in vitro. *Exp Cell Res.* 49(1):181-93.
- Comper WD, Laurent TC. (1978) Physiological function of connective tissue polysaccharides. *Physiol Rev.* 58(1):255-315.
- Contreras JL, Smyth CA, Eckstein C, Bilbao G, Thompson JA, Young CJ, Eckhoff DE. (2003) Peripheral mobilization of recipient bone marrow-derived endothelial progenitor cells enhances pancreatic islet revascularization and engraftment after intraportal transplantation. *Surgery* 134(2):390-8.
- Cottler-Fox MH, Lapidot T, Petit I, Kollet O, DiPersio JF, Link D, Devine S. (2003) Stem cell mobilization. *Hematology Am Soc Hematol Educ Program.* 419-37.
- Croop JM, Cooper R, Fernandez C, Graves V, Kreissman S, Hanenberg H, Smith FO, Williams DA. (2001) Mobilization and collection of peripheral blood CD34+ cells from patients with Fanconi anemia. *Blood.* 98(10):2917-21.
- Dasgupta C, Escobar-Poni B, Shah M, Duncan J, Fletcher WH. (1999) Misregulation of connexin43 gap junction channels and congenital heart defects. *Novartis Found Symp.* 219:212-21.
- Deasy BM, Qu-Peterson Z, Greenberger JS, Huard J. (2002) Mechanisms of muscle stem cell expansion with cytokines. *Stem Cells.* 20(1):50-60.

- De Clercq E. (2005) Potential clinical applications of the CXCR4 antagonist bicyclam AMD3100. *Mini Rev Med Chem.* 5(9):805-24.
- Diller GP, van Eijl S, Okonko DO, Howard LS, Ali O, Thum T, Wort SJ, Bédard E, Gibbs JS, Bauersachs J, Hobbs AJ, Wilkins MR, Gatzoulis MA, Wharton J. (2008) Circulating endothelial progenitor cells in patients with Eisenmenger syndrome and idiopathic pulmonary arterial hypertension. *Circulation* 117(23):3020-30.
- Docherty K. (2009) Pancreatic stellate cells can form new beta-like cells. *Biochem J.* 421(2): e1-4.
- Dos Santos F, Andrade PZ, Eibes G, da Silva CL, Cabral JM. (2011) Ex Vivo Expansion of Human Mesenchymal Stem Cells on Microcarriers. *Methods Mol Biol.* 698:189-198.
- Drapeau C, Antarr D, Ma H, Yang Z, Tang L, Hoffman RM, Schaeffer DJ. (2010) Mobilization of bone marrow stem cells with StemEnhance improves muscle regeneration in cardiotoxin-induced muscle injury. *Cell Cycle* 9(9):1819-23.
- Dunac A, Frelin C, Popolo-Blondeau M, Chatel M, Mahagne MH, Philip PJ. (2007) Neurological and functional recovery in human stroke are associated with peripheral blood CD34+ cell mobilization. *J Neurol.* 2007 Mar;254(3):327-32.
- Eizawa T, Murakami Y, Matsui K, Takahashi M, Muroi K, Amemiya M, Takano R, Kusano E, Shimada K, Ikeda U. (2003) Circulating endothelial progenitor cells are reduced in hemodialysis patients. *Curr Med Res Opin.* 19(7):627-33.
- Ellis SG, Penn MS, Bolwell B, Garcia M, Chacko M, Wang T, Brezina KJ, McConnell G, Topol EJ. (2006) Granulocyte colony stimulating factor in patients with large acute myocardial infarction: results of a pilot dose-escalation randomized trial. *Am Heart J.* 152(6):1051.e9-14.
- Fadini GP, de Kreutzenberg S, Agostini C, Boscaro E, Tiengo A, Dimmeler S, Avogaro A (2009) *Low CD34+ cell count and metabolic syndrome synergistically increase the risk of adverse outcomes. Atherosclerosis* 2009; 207: 213– 219
- Fadini GP, Boscaro E, de Kreutzenberg S, Agostini C, Seeger F, Dimmeler S, Zeiher A, Tiengo A, Avogaro A. (2010) Time course and mechanisms of circulating progenitor cell reduction in the natural history of type 2 diabetes. *Diabetes Care.* 2010 May;33(5):1097-102.
- Fallon J, S Reid, R Kinyamu, I Opole, R Opole, J Baratta, M Korc, TL Endo, A Duong, G Nguyen, M Karkehabadhi, D Twardzik, S Patel and S Loughlin. (2000). In vivo induction of massive proliferation, directed migration, and differentiation of neural cells in the adult mammalian brain. *PNAS* 96:14686–14691.
- Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F. (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. *Science.* 279(5356):1528-30.
- Fibbe WE, Pruijt JF, Velders GA, et al. (1999) Biology of IL-8-induced stem cell mobilization. *Ann N Y Acad Sci.* 872:71-82.
- Fox A, Smythe J, Fisher N, Tyler MP, McGruther DA, Watt SM, Harris AL. (2008) Mobilization of endothelial progenitor cells into the circulation in burned patients. *Br J Surg.* 95(2):244-51
- Fraser JR, Laurent TC, Laurent UB. (1997) Hyaluronan: its nature, distribution, functions and turnover. *J Intern Med.* 242(1):27-33.

- Friedl P, Maaser K, Klein CE, Niggemann B, Krohne G, Zanker KS. Migration of highly aggressive MV3 melanoma cells in 3-dimensional collagen lattices results in local matrix reorganization and shedding of alpha2 and beta1 integrins and CD44. *Cancer Res.*57:2061-2070.
- Fukuda S, Bian H, King AG, Pelus LM. (2007) The chemokine GRObeta mobilizes early hematopoietic stem cells characterized by enhanced homing and engraftment. *Blood* 110(3):860-9.
- Fukuhara S, Tomita S, Nakatani T, Ohtsu Y, Ishida M, Yutani C, Kitamura S. (2004) G-CSF promotes bone marrow cells to migrate into infarcted mice heart, and differentiate into cardiomyocytes. *Cell Transplant.* 13(7-8):741-8
- Gao X, Song L, Shen K, Wang H, Niu W, Qin X. (2008) Transplantation of bone marrow derived cells promotes pancreatic islet repair in diabetic mice. *Biochem Biophys Res Commun.* 371(1):132-7.
- Gnecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, Noiseux N, Zhang L, Pratt RE, Ingwall JS, Dzau VJ. (2005) Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. *Nat Med.* 11(4):367-8.
- Gnecchi M, He H, Noiseux N, Liang OD, Zhang L, Morello F, Mu H, Melo LG, Pratt RE, Ingwall JS, Dzau VJ. (2006) Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. *FASEB J.* 20(6):661-9.
- Gordon MK, Sher D, Karrison T, Kebriaei P, Chuang K, Zhang Y, McDonnell D, Artz A, Godley L, Odenike O, Rich E, Michaelis L, Thirman MJ, Wickrema A, van Besien K, Larson RA, Stock W. (2008) Successful autologous stem cell collection in patients with chronic myeloid leukemia in complete cytogenetic response, with quantitative measurement of BCR-ABL expression in blood, marrow, and apheresis products. *Leuk Lymphoma.* 49(3):531-7.
- Greco SJ, Zhou C, Ye JH, Rameshwar P. (2008) A method to generate human mesenchymal stem cell-derived neurons which express and are excited by multiple neurotransmitters. *Biol Proced Online.* 10:90-101.
- Grisar J, Aletaha D, Steiner CW, Kapral T, Steiner S, Seidinger D, Weigel G, Schwarzingler I, Wolozczuk W, Steiner G, Smolen JS. (2005) Depletion of endothelial progenitor cells in the peripheral blood of patients with rheumatoid arthritis. *Circulation.* 111(2):204-11.
- Gronthos S, Arthur A, Bartold PM, Shi S. (2011) A method to isolate and culture expand human dental pulp stem cells. *Methods Mol Biol.* 698:107-21.
- Hahn JY, Cho HJ, Kang HJ, Kim TS, Kim MH, Chung JH, Bae JW, Oh BH, Park YB, Kim HS. (2008) Pre-treatment of mesenchymal stem cells with a combination of growth factors enhances gap junction formation, *J Am Coll Cardiol.* 51(9):933-43.
- Hasegawa Y, Ogihara T, Yamada T, Ishigaki Y, Imai J, Uno K, Gao J, Kaneko K, Ishihara H, Sasano H, Nakauchi H, Oka Y, Katagiri H. (2007) Bone marrow (BM) transplantation promotes beta-cell regeneration after acute injury through BM cell mobilization. *Endocrinology.* 148(5):2006-15.

- Hatch HM, Zheng D, Jorgensen ML, Petersen BE. (2002) SDF-1alpha/CXCR4: a mechanism for hepatic oval cell activation and bone marrow stem cell recruitment to the injured liver of rats. *Cloning Stem Cells*. 4(4):339-51.
- Hayakawa J, Migita M, Ueda T, Shimada T, Fukunaga Y. (2003) Generation of a chimeric mouse reconstituted with green fluorescent protein-positive bone marrow cells: a useful model for studying the behavior of bone marrow cells in regeneration in vivo. *Int J Hematol*. 77(5):456-62.
- Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, Hackett NR, Crystal RG, Besmer P, Lyden D, Moore MA, Werb Z, Rafii S. (2002) Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell*. 109:625-637.
- Hennemann B, Ickenstein G, Sauerbruch S, Luecke K, Haas S, Horn M, Andreesen R, Bogdahn U, Winkler J. (2008) Mobilization of CD34+ hematopoietic cells, colony-forming cells and long-term culture-initiating cells into the peripheral blood of patients with an acute cerebral ischemic insult. *Cytotherapy*. 10(3):303-11.
- Henschler R, Deak E, Seifried E. (2008) Homing of Mesenchymal Stem Cells. *Transfus Med Hemother*. 35(4):306-312.
- Herbrig K, Haensel S, Oelschlaegel U, Pistrosch F, Foerster S, Passauer J. (2006) Endothelial dysfunction in patients with rheumatoid arthritis is associated with a reduced number and impaired function of endothelial progenitor cells. *Ann Rheum Dis*. 65(2):157-63.
- Herbrig K, Pistrosch F, Oelschlaegel U, Wichmann G, Wagner A, Foerster S, Richter S, Gross P, Passauer J. (2004) Increased total number but impaired migratory activity and adhesion of endothelial progenitor cells in patients on long-term hemodialysis. *Am J Kidney Dis*. 44(5):840-9.
- Hess DC, Hill WD, Martin-Studdard A, Carroll J, Brailer J, Carothers J. (2002) Bone marrow as a source of endothelial cells and NeuN-expressing cells after stroke. *Stroke* 33(5):1362-8.
- Hisashi Y, Tomita S, Nakatani T, Fukuhara S, Yutani C, Kitamura S. (2004) Granulocyte-colony stimulating factor enhanced the recruitment of bone marrow cells into the heart: time course evaluation of phenotypic differentiation in the doxorubicin-induced cardiomyopathic model. *Jpn J Thorac Cardiovasc Surg*. 52(10):451-5.
- Hoehn M, Küstermann E, Blunk J, Wiedermann D, Trapp T, Wecker S, Föcking M, Arnold H, Hescheler J, Fleischmann BK, Schwindt W, Bührle C. (2002) Monitoring of implanted stem cell migration in vivo: a highly resolved in vivo magnetic resonance imaging investigation of experimental stroke in rat. *Proc Natl Acad Sci U S A*. 99(25):16267-72.
- Hofmann NA, Reinisch A, Strunk D. (2009) Isolation and large scale expansion of adult human endothelial colony forming progenitor cells. *J Vis Exp*. 28:(32).
- Hwang JH, Kim SW, Park SE, Yun HJ, Lee Y, Kim S, Jo DY. (2006) Overexpression of stromal cell-derived factor-1 enhances endothelium-supported transmigration, maintenance, and proliferation of hematopoietic progenitor cells. *Stem Cells Dev*. 15(2):260-8.

- Ince H, Petzsch M, Kleine HD, Eckard H, Rehders T, Burska D, Kische S, Freund M, Nienaber CA. (2005) Prevention of left ventricular remodeling with granulocyte colony-stimulating factor after acute myocardial infarction: final 1-year results of the Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction by Granulocyte Colony-Stimulating Factor (FIRSTLINE-AMI) Trial. *Circulation* 112(9 Suppl):I73-80.
- Insausti CL, Blanquer M, Meseguer L, López-Martínez MC, Ferez X, Rodriguez FJ, Cabañas V, Funes C, Nicolás FJ, Majado MJ, Moraleda JM. Isolation and characterization of mesenchymal stem cells from the fat layer on the density gradient separated bone marrow. *Stem Cells Dev.* 2011 Apr 19. [Epub ahead of print]
- Ivanovic Z, Duchez P, Chevaleyre J, Vlaski M, Lafarge X, Dazey B, Robert-Richard E, Mazurier F, Boiron JM. (2011) Clinical-scale cultures of cord blood CD34+ cells to amplify committed progenitors and maintain stem cell activity. *Cell Transplant.* 2011 Feb 3. [Epub ahead of print]
- Iwaguro H, Yamaguchi J, Kalka C, Murasawa S, Masuda H, Hayashi S, Silver M, Li T, Isner JM, Asahara T. (2002) Endothelial progenitor cell vascular endothelial growth factor gene transfer for vascular regeneration. *Circulation* 105:732-738.
- Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Michael LH, Hirschi KK, Goodell MA. (2001) Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest.* 107(11):1395-402.
- Jang YY, Collector MI, Baylin SB, Diehl AM, Sharkis SJ. (2004) Hematopoietic stem cells convert into liver cells within days without fusion. *Nat Cell Biol.* 6(6):532-9.
- Janowska-Wieczorek A, Marquez LA, Nabholz JM, Cabuhat ML, Montaña J, Chang H, Rozmus J, Russell JA, Edwards DR, Turner AR. (1999) Growth factors and cytokines upregulate gelatinase expression in bone marrow CD34(+) cells and their transmigration through reconstituted basement membrane. *Blood.* 93:3379-3390.
- Janowska-Wieczorek A, Marquez LA, Dobrowsky A, Ratajczak MZ, Cabuhat ML. (2000) Differential MMP and TIMP production by human marrow and peripheral blood CD34- cells in response to chemokines. *Exp Hematol* 28:1274-1285.
- Jensen GS, Hart AN, Zaske LA, Drapeau C, Gupta N, Schaeffer DJ, Cruickshank JA. (2007) Mobilization of human CD34+ CD133+ and CD34+ CD133(-) stem cells in vivo by consumption of an extract from *Aphanizomenon flos-aquae*-related to modulation of CXCR4 expression by an L-selectin ligand? *Cardiovasc Revasc Med.* 8(3):189-202.
- Jin FY, Qiu LG, Li QC, Meng HX, Wang YF, Yu Z, Li Q, Han JL. (2006) The effect of matrix metalloproteinase-9 in granulocyte colony stimulation factor-induced stem cell mobilization. *Zhonghua Yi Xue Za Zhi.* 86(42):2966-70.
- Jo DY, Rafii S, Hamada T, Moore MA. (2000) Chemotaxis of primitive hematopoietic cells in response to stromal cell-derived factor-1. *J Clin Invest.* 105(1):101-11.
- Junhui Z, Xingxiang W, Guosheng F, Yunpeng S, Furong Z, Junzhu C. (2008) Reduced number and activity of circulating endothelial progenitor cells in patients with idiopathic pulmonary arterial hypertension. *Respir Med.* 102(7):1073-9.
- Kajstura J, Leri A, Finato N, Di Loreto C, Beltrami CA, Anversa P. (1998) Myocyte proliferation in end-stage cardiac failure in humans. *Proc Natl Acad Sci USA* 95:8801-8805.

- Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Tsutsumi Y, Ozono R, Masaki H, Mori Y, Iba O, Tateishi E, Kosaki A, Shintani S, Murohara T, Imaizumi T, Iwasaka T. (2001) Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation*. 104(9):1046-52.
- Kan I, Melamed E, Offen D. (2005) Integral therapeutic potential of bone marrow mesenchymal stem cells. *Curr Drug Targets*. 6(1):31-41.
- Kang S, Yang Y, Li CJ, Gao R. (2007) Effectiveness and tolerability of administration of granulocyte colony-stimulating factor on left ventricular function in patients with myocardial infarction: a meta-analysis of randomized controlled trials. *Clin Ther*. 2007 Nov;29(11):2406-18.
- Karra R, Wu SM. (2008) Multipotent stem cells in cardiac regenerative therapy. *Regen Med*. 3(2):189-98.
- Kawada H, Takizawa S, Takanashi T, Morita Y, Fujita J, Fukuda K, Takagi S, Okano H, Ando K, Hotta T. (2006) Administration of hematopoietic cytokines in the subacute phase after cerebral infarction is effective for functional recovery facilitating proliferation of intrinsic neural stem/progenitor cells and transition of bone marrow-derived neuronal cells. *Circulation*. 113(5):701-10.
- Keilhoff G, Stang F, Goehl A, Wolf G, Fansa H. (2006) Transdifferentiated mesenchymal stem cells as alternative therapy in supporting nerve regeneration and myelination. *Cell Mol Neurobiol*. 26(7-8):1235-52.
- King AG, Horowitz D, Dillon SB, Levin R, Farese AM, MacVittie TJ, Pelus LM. (2001) Rapid mobilization of murine hematopoietic stem cells with enhanced engraftment properties and evaluation of hematopoietic progenitor cell mobilization in rhesus monkeys by a single injection of SB-251353, a specific truncated form of the human CXC chemokine GRObeta. *Blood*. 97(6):1534-42.
- Kinnaird T, Stabile E, Burnett MS, Shou M, Lee CW, Barr S, Fuchs S, Epstein SE. (2004) Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. *Circulation*. 109(12):1543-9.
- Kleeberger W, Versmold A, Rothämel T, Glöckner S, Bredt M, Haverich A, Lehmann U, Kreipe H. (2003) Increased chimerism of bronchial and alveolar epithelium in human lung allografts undergoing chronic injury. *Am J Pathol*. 162(5):1487-94.
- Klein JD, Fauza DO. (2011) Amniotic and placental mesenchymal stem cell isolation and culture. *Methods Mol Biol*. 698:75-88.
- Kolbe M, Dohle E, Katerla D, Kirkpatrick CJ, Fuchs S. (2010) Enrichment of outgrowth endothelial cells in high and low colony-forming cultures from peripheral blood progenitors. *Tissue Eng Part C Methods*. 16(5):877-86.
- Kollet O, Shivtiel S, Chen YQ, Suriawinata J, Thung SN, Dabeva MD, Kahn J, Spiegel A, Dar A, Samira S, Goichberg P, Kalinkovich A, Arenzana-Seisdedos F, Nagler A, Hardan I, Revel M, Shafritz DA, Lapidot T. (2003) HGF, SDF-1, and MMP-9 are involved in stress-induced human CD34+ stem cell recruitment to the liver. *J Clin Invest*. 112(2):160-9.

- Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, and Sharkis SJ (2001) Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 105:369-377
- Kucia M, Ratajczak J, Reza R, Janowska-Wieczorek A, Ratajczak MZ.. (2004) Tissue-specific muscle, neural and liver stem/progenitor cells reside in the bone marrow, respond to an SDF-1 gradient and are mobilized into peripheral blood during stress and tissue injury. *Blood Cells Mol Dis.* 32(1):52-57.
- Laflamme MA, Myerson D, Saffitz JE, Murry CE. (2002) Evidence for cardiomyocyte repopulation by extracardiac progenitors in transplanted human hearts. *Circ Res.* 90(6):634-40.
- Lapid K, Vagima Y, Kollet O, Lapidot T. (2009) Egress and mobilization of hematopoietic stem and progenitor cells. *StemBook*. Cambridge (MA): Harvard Stem Cell Institute; 2008-.2009 Mar 15.
- Lapidot T and Petit I. (2002) Current understanding of stem cell mobilization: the roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. *Exp Hematol.* 30(9):973-81.
- Lazarini F, Tham TN, Casanova P, Arenzana-Seisdedos F, Dubois-Dalq M. (2003) Role of the alpha-chemokine stromal cell-derived factor (SDF-1) in the developing and mature central nervous system. *Glia* 2003;42:139-148.
- Lee DY, Cho TJ, Kim JA, Lee HR, Yoo WJ, Chung CY, Choi IH. (2008) Mobilization of endothelial progenitor cells in fracture healing and distraction osteogenesis *Bone.* 42(5):932-41.
- Lee HJ, Kim KS, Park IH, Kim SU (2007) Human neural stem cells over-expressing VEGF provide neuroprotection, angiogenesis and functional recovery in mouse stroke model. *PLoS ONE* 2(1): e156.
- Lee KD, Kuo TK, Whang-Peng J, Chung YF, Lin CT, Chou SH, Chen JR, Chen YP and Lee OK. (2004) In vitro hepatic differentiation of human mesenchymal stem cells. *Hepatology* 40:1275-1284.
- Lee ST, Chu K, Jung KH, Ko SY, Kim EH, Sinn DI, Lee YS, Lo EH, Kim M, Roh JK. (2005) Granulocyte colony-stimulating factor enhances angiogenesis after focal cerebral ischemia. *Brain Res.* 1058(1-2):120-128.
- Lee ST, Chu K, Jung KH, Kim DH, Kim EH, Choe VN, Kim JH, Im WS, Kang L, Park JE, Park HJ, Park HK, Song EC, Lee SK, Kim M, Roh JK. (2008) Decreased number and function of endothelial progenitor cells in patients with migraine. *Neurology.* 70(17):1510-7.
- Lemery SJ, Hsieh MM, Smith A, Rao S, Khuu HM, Theresa D, Viano JM, Cook L, Goodwin R, Boss C, Calandra G, Geller N, Tisdale J, Childs R. (2011) A pilot study evaluating the safety and CD34+ cell mobilizing activity of escalating doses of plerixafor in healthy volunteers. *Br J Haematol.* 153(1):66-75.
- Leone AM, Rutella S, Bonanno G, Contemi AM, de Ritis DG, Giannico MB, Rebuzzi AG, Leone G, Crea F. (2006) Endogenous G-CSF and CD34+ cell mobilization after acute myocardial infarction. *Int J Cardiol.* 111(2):202-8.
- Leone AM, Galiuto L, Garramone B, Rutella S, Giannico MB, Brugaletta S, Perfetti M, Liuzzo G, Porto I, Burzotta F, Niccoli G, Biasucci LM, Leone G, Rebuzzi AG, Crea F. (2007)

- Usefulness of granulocyte colony-stimulating factor in patients with a large anterior wall acute myocardial infarction to prevent left ventricular remodeling (the rigenera study). *Am J Cardiol.* 100(3):397-403.
- Li Y, Chen J, Chen XG, Wang L, Gautam SC, Xu YX, Katakowski M, Zhang LJ, Lu M, Janakiraman N, Chopp M. (2002) Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. *Neurology.* 59(4):514-23.
- Lima C, Escada P, Pratas-Vital J, Branco C, Arcangeli CA, Lazzeri G, Maia CA, Capucho C, Hasse-Ferreira A, Peduzzi JD. (2010) Olfactory mucosal autografts and rehabilitation for chronic traumatic spinal cord injury. *Neurorehabil Neural Repair.* 24(1):10-22.
- Loewenstein WR, Rose B. (1992) The cell-cell channel in the control of growth. *Semin Cell Biol.* 3(1):59-79.
- Mannello F, Tonti GA, Bagnara GP, Papa S. (2006) Role and function of matrix metalloproteinases in the differentiation and biological characterization of mesenchymal stem cells. *Stem Cells.* 24(3):475-81.
- Mansilla E, Marín GH, Drago H, Sturla F, Salas E, Gardiner C, Bossi S, Lamonega R, Guzmán A, Nuñez A, Gil MA, Piccinelli G, Ibar R, Soratti C. (2006) Bloodstream cells phenotypically identical to human mesenchymal bone marrow stem cells circulate in large amounts under the influence of acute large skin damage: new evidence for their use in regenerative medicine. *Transplant Proc.* 38(3):967-9.
- Marchesi C, Belicchi M, Meregalli M, Farini A, Cattaneo A, Parolini D, Gavina M, Porretti L, D'Angelo MG, Bresolin N, Cossu G, Torrente Y. (2008) Correlation of circulating CD133+ progenitor subclasses with a mild phenotype in Duchenne muscular dystrophy patients. *PLoS ONE.* 3(5):e2218.
- Magavi SS and JD Macklis. (2002). Immunocytochemical analysis of neuronal differentiation. *Meth Mol Biol* 198:291-297.
- Mathews V, Hanson PT, Ford E, Fujita J, Polonsky KS, Graubert TA. (2004) Recruitment of bone marrow-derived endothelial cells to sites of pancreatic beta-cell injury. *Diabetes* 53(1):91-8.
- Matsumoto T, Mifune Y, Kawamoto A, Kuroda R, Shoji T, Iwasaki H, Suzuki T, Oyamada A, Horii M, Yokoyama A, Nishimura H, Lee SY, Miwa M, Doita M, Kurosaka M, Asahara T. (2008) Fracture induced mobilization and incorporation of bone marrow-derived endothelial progenitor cells for bone healing. *J Cell Physiol.* 215(1):234-42
- Mezey E, Key S, Vogelsang G, Szalayova I, Lange GD and Crain G. (2003). Transplanted bone marrow generates new neurons in human brains. *Proc. Natl. Acad. Sci. USA* 100, 1364-1369.
- Middleton J, Neil S, Wintle J, Clark-Lewis I, Moore H, Lam C, Auer M, Hub E, Rot A. (1997) Transcytosis and surface presentation of IL-8 by venular endothelial cells. *Cell.* 91(3):385-95.
- Mirotsoy M, Jayawardena TM, Schmeckpeper J, Gneccchi M, Dzau VJ. (2011) Paracrine mechanisms of stem cell reparative and regenerative actions in the heart. *J Mol Cell Cardiol.* 50(2):280-9.

- Mishra SK, Braun N, Shukla V, Füllgrabe M, Schomerus C, Korf HW, Gachet C, Ikehara Y, Sévigny J, Robson SC, Zimmermann H. (2006) Extracellular nucleotide signaling in adult neural stem cells: synergism with growth factor-mediated cellular proliferation. *Development*. 133(4):675-84.
- Moonen JR, de Leeuw K, van Seijen XJ, Kallenberg CG, van Luyn MJ, Bijl M, Harmsen MC. (2007) Reduced number and impaired function of circulating progenitor cells in patients with systemic lupus erythematosus. *Arthritis Res Ther*. 2007;9(4):R84.
- Müller P, Pfeiffer P, Koglin J, Schäfers HJ, Seeland U, Janzen I, Urbchat S, Böhm M. (2002) Cardiomyocytes of noncardiac origin in myocardial biopsies of human transplanted hearts. *Circulation*. 106(1):31-5.
- Nakatomi H, T Kuriu, S Okabe, S Yamamoto, O Hatano, N Kawahara, A Tamura, T Kirino and M Nakafuku. (2002) Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. *Cell* 110:429-441.
- Neijssen J, Herberts C, Drijfhout JW, Reits E, Janssen L, Neefjes J. (2005) Cross-presentation by intercellular peptide transfer through gap junctions. *Nature*. 434(7029):83-8.
- Neuss S, Becher E, Wöltje M, Tietze L, Jahnen-Dechent W. (2004) Functional expression of HGF and HGF receptor/c-met in adult human mesenchymal stem cells suggests a role in cell mobilization, tissue repair, and wound healing. *Stem Cells* 22:405- 414.
- Nygren JM, Jovinge S, Breitbach M, Säwén P, Röhl W, Hescheler J, Taneera J, Fleischmann BK, Jacobsen SE. (2004) Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. *Nat Med*.10(5):494-501.
- Okamoto I, Kawano Y, Tsuiki H, Sasaki J, Nakao M, Matsumoto M, Suga M, Ando M, Nakajima M, Saya H. (1999) CD44 cleavage induced by a membrane-associated metalloprotease plays a critical role in tumor cell migration. *Oncogene*. 18:1435-1446.
- Orlic D., Kajstura J., Chimenti S., Jakoniuk I, Pickel J., McKay R., Nadal-Ginard B, Bodine D. M., Leri A. & Anversa P. (2001a) Bone marrow cells regenerate infarcted myocardium. *Nature (London)* 410, 701-705.
- Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. (2001b) Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci U S A*. 98(18):10344-9.
- Osada T, Watanabe M, Hasuo A, Imai M, Suyama K, Sakai D, Kawada H, Matsumae M, Mochida J. (2010) Efficacy of the coadministration of granulocyte colony-stimulating factor and stem cell factor in the activation of intrinsic cells after spinal cord injury in mice. *J Neurosurg Spine*. 13(4):516-23.
- Paczkowska E, Larysz B, Rzeuski R, Karbicka A, Jałowiński R, Kornacewicz-Jach Z, Ratajczak MZ, Machaliński B. (2005) Human hematopoietic stem/progenitor-enriched CD34(+) cells are mobilized into peripheral blood during stress related to ischemic stroke or acute myocardial infarction. *Eur J Haematol*. 75(6):461-7.
- Pavlichenko N, Sokolova I, Vijde S, Shvedova E, Alexandrov G, Krouglyakov P, Fedotova O, Gilerovich EG, Polyntsev DG, Otellin VA Mesenchymal stem cells transplantation

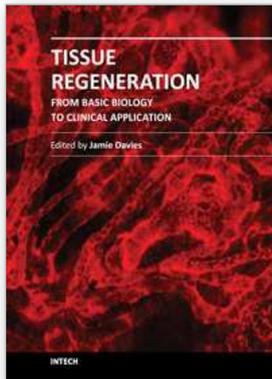
- could be beneficial for treatment of experimental ischemic stroke in rats. *Brain Res.* 2008 Jul 14.
- Peled A, Grabovsky V, Habler L, Sandbank J, Arenzana-Seisdedos F, Petit I, Ben-Hur H, Lapidot T, Alon R. (1999) The chemokine SDF-1 stimulates integrin-mediated arrest of CD34(+) cells on vascular endothelium under shear flow. *J Clin Invest.* 104(9):1199-211.
- Peled A, Kollet O, Ponomaryov T, Petit I, Frantza S, Grabovsky V, Slav MM, Nagler A, Lider O, Alon R, Zipori D, Lapidot T. (2000) The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34(+) cells: role in transendothelial/stromal migration and engraftment of NOD/SCID mice. *Blood.* 95(11):3289-96.
- Pereira RF, O'Hara MD, Laptev AV, Halford KW, Pollard MD, Class R, Simon D, Livezey K and Prockop DJ. (1998) Marrow stromal cells as a source of progenitor cells for nonhematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta. *Proc. Natl. Acad. Sci. USA* 95, 1142-1147.
- Peterson DA. (2002). Stem cells in brain plasticity and repair. *Curr Opin Pharmacol* 2:34-42.
- Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, Habler L, Ponomaryov T, Taichman RS, Arenzana-Seisdedos F, Fujii N, Sandbank J, Zipori D, Lapidot T. (2003) G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat Immunol.* 3(7):687-94.
- Pineault N, Cortin V, Boyer L, Garnier A, Robert A, Thérien C, Roy DC. (2011) Individual and synergistic cytokine effects controlling the expansion of cord blood CD34(+) cells and megakaryocyte progenitors in culture. *Cytotherapy.* 13(4):467-80.
- Quaini F, Urbanek K, Beltrami AP, Finato N, Beltrami CA, Nadal-Ginard B, Kajstura J, Leri A, Anversa P. (2002) Chimerism of the transplanted heart. *N Engl J Med.* 346(1):5-15.
- Ratajczak MZ, Majka M, Kucia M, Drukala J, Pietrkowski Z, Peiper S, Janowska-Wieczorek A. (2003) Expression of functional CXCR4 by muscle satellite cells and secretion of SDF-1 by muscle-derived fibroblasts is associated with the presence of both muscle progenitors in bone marrow and hematopoietic stem/progenitor cells in muscles. *Stem Cells.* 21(3):363-71.
- Reed RK, Lilja K, Laurent TC. (1988) Hyaluronan in the rat with special reference to the skin. *Acta Physiol Scand.* 134(3):405-11.
- Ries C, Egea V, Karow M, Kolb H, Jochum M, Neth P. (2007) MMP-2, MT1-MMP, and TIMP-2 are essential for the invasive capacity of human mesenchymal stem cells: differential regulation by inflammatory cytokines. *Blood.* 109(9):4055-63.
- Ripa RS, Kastrup J. (2008) G-CSF therapy with mobilization of bone marrow stem cells for myocardial recovery after acute myocardial infarction--a relevant treatment? *Exp Hematol.* 36(6):681-6.
- Rustom A, Saffrich R, Markovic I, Walther P, Gerdes HH. (2004) Nanotubular highways for intercellular organelle transport. *Science.* 303(5660):1007-10.
- Sanchez-Ramos JR. (2002) Neural cells derived from adult bone marrow and umbilical cord blood. *J Neurosci Res.* 69(6):880-93

- Schioppa T, Uranchimeg B, Saccani A, Biswas SK, Doni A, Rapisarda A, Bernasconi S, Saccani S, Nebuloni M, Vago L, Mantovani A, Melillo G, Sica A. (2003) Regulation of the chemokine receptor CXCR4 by hypoxia. *J Exp Med* 2003;198:1391-1402.
- Schmidt W and KG Reymann. (2002). Proliferating cells differentiate into neurons in the hippocampal CA1 region of gerbils after global cerebral ischemia. *Neurosci Lett* 334:153-156.
- Schrader AJ, Lechner O, Templin M, Dittmar KE, Machtens S, Mengel M, Probst-Kepper M, Franzke A, Wollensak T, Gatzlaff P, Atzpodien J, Buer J, Lauber J. (2002) CXCR4/CXCL12 expression and signalling in kidney cancer. *Br J Cancer*. 86(8):1250-6.
- Schwartz Y and Kornowski R. (2003) Autologous stem cells for functional myocardial repair. *Heart Fail Rev*. 8(3):237-45.
- Sesti C, Hale SL, Lutzko C, Kloner RA. (2005) Granulocyte colony-stimulating factor and stem cell factor improve contractile reserve of the infarcted left ventricle independent of restoring muscle mass. *J Am Coll Cardiol*. 46(9):1662-9.
- Shimoda K, Okamura S, Haarada N, Kondo S, Niho Y. (1993) Identification of a functional receptor for granulocyte colony-stimulating factor on platelets. *J Clin Invest*. 91:1310-1313.
- Shintani S, Murohara T, Ikeda H, Ueno T, Honma T, Katoh A, Sasaki K, Shimada T, Oike Y, Imaizumi T. (2001) Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. *Circulation*. 103(23):2776-9.
- Shyu WC, Lin SZ, Yang HI, Tzeng YS, Pang CY, Yen PS, Li H. (2004) Functional recovery of stroke rats induced by granulocyte colony-stimulating factor-stimulated stem cells. *Circulation* 110(13):1847-54.
- Six I, Gasan G, Mura E, Bordet R. (2003) Beneficial effect of pharmacological mobilization of bone marrow in experimental cerebral ischemia. *Eur J Pharmacol*. 458(3):327-8.
- Snykers S, De Kock J, Tamara V, Rogiers V. (2011) Hepatic differentiation of mesenchymal stem cells: in vitro strategies. *Methods Mol Biol*. 698:305-14.
- Soonpaa MH and Field LJ. (1998) Survey of studies examining mammalian cardiomyocyte DNA synthesis. *Am Heart Assoc* 1998;83:15-26.
- Spees JL, Olson SD, Ylostalo J, Lynch PJ, Smith J, Perry A, Peister A, Wang MY, Prockop DJ. (2003) Differentiation, cell fusion, and nuclear fusion during ex vivo repair of epithelium by human adult stem cells from bone marrow stroma. *Proc Natl Acad Sci U S A*. 100(5):2397-402.
- Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, Schümichen C, Nienaber CA, Freund M, Steinhoff G. (2003) Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet*. 361(9351):45-6.
- Stout CL, Ashley DW, Morgan JH 3rd, Long GF, Collins JA, Limnios JI, Lochner F, McCommon G, Hixson D, Black AC Jr, Young HE. (2007) Primitive stem cells residing in the skeletal muscle of adult pigs are mobilized into the peripheral blood after trauma. *Am Surg*. 73(11):1106-10.
- Suratt BT, Cool CD, Serls AE, Chen L, Varella-Garcia M, Shpall EJ, Brown KK, Worthen GS. (2003) Human pulmonary chimerism after hematopoietic stem cell transplantation. *Am J Respir Crit Care Med*. 168(3):318-22.

- Swenson ES, Kuwahara R, Krause DS, Theise ND. (2008) Physiological variations of stem cell factor and stromal-derived factor-1 in murine models of liver injury and regeneration. *Liver Int.* 28(3):308-18
- Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Magner M, Isner JM, Asahara T. (1999) Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med.* 5(4):434-8.
- Terada N, Hamazaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y, Meyer EM, Morel L, Petersen BE, Scott EW. (2002) Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature.* 416(6880):542-5.
- Theise ND, Nimmakayalu M, Gardner R, Illei PB, Morgan G, Teperman L, Henegariu O, Krause DS. (2000) Liver from bone marrow in humans. *Hepatology.* 32(1):11-6.
- Tirino V, Paino F, d'Aquino R, Desiderio V, De Rosa A, Papaccio G. (2011) Methods for the Identification, Characterization and Banking of Human DPSCs: Current Strategies and Perspectives. *Stem Cell Rev.* Feb 12. [Epub ahead of print]
- Tomita M, Adachi Y, Yamada H, Takahashi K, Kiuchi K, Oyaizu H, Ikebukuro K, Kaneda H, Matsumura M, Ikehara S. (2002) Bone marrow-derived stem cells can differentiate into retinal cells in injured rat retina. *Stem Cells.* 20(4):279-83.
- Tomoda H and Aoki N. (2003) Bone marrow stimulation and left ventricular function in acute myocardial infarction. *Clin. Cardiol.* 26:455-457
- Trzaska KA, Rameshwar P. (2011) Dopaminergic Neuronal Differentiation Protocol for Human Mesenchymal Stem Cells. *Methods Mol Biol.* 698:295-303.
- Tsagias N, Koliakos I, Lappa M, Karagiannis V, Koliakos GG. (2011) Placenta perfusion has hematopoietic and mesenchymal progenitor stem cell potential. *Transfusion.* 51(5):976-85.
- Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. (2003) Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. *Lancet.* 361(9351):47-9.
- Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, Zeiher AM, and Dimmeler S. (2001) Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res.* 89:e1-e7.
- Vasania VS, Prasad P, Gill RK, Mehta A, Viswanathan C, Sarang S, Majumdar AS. (2010) Molecular and cellular characterization of expanded and cryopreserved human limbal epithelial stem cells reveal unique immunological properties. *Exp Eye Res.* 92(1):47-56.
- Vassilopoulos G, Wang PR, Russell DW. (2003) Transplanted bone marrow regenerates liver by cell fusion. *Nature.* 422(6934):901-4.
- Viktorov IV, Savchenko EA, Ukhova OV, Alekseyeva NY, Chekhonin VP. (2006) Multipotent stem and progenitor cells of the olfactory epithelium. *Bull Exp Biol Med.* 142(4):495-502.
- Voermans C, Rood PM, Hordijk PL, Gerritsen WR, van der Schoot CE. (2000) Adhesion molecules involved in transendothelial migration of human hematopoietic progenitor cells. *Stem Cells.* 18(6):435-43.
- Voltarelli JC, Couri CEB, Stracieri ABPL, Oliveira MC, Moraes DA, Pieroni F, Coutinho M, Malmegrim KCR, Foss-Freitas MC, Simoes BP, Foss MC, Squiers E, and Burt RK.

- (2007) Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed Type 1 diabetes mellitus. *JAMA*. 297(14):1568-1576.
- Wang J, Wang X, Sun Z, Wang X, Yang H, Shi S, Wang S. (2011) Stem cells from human-exfoliated deciduous teeth can differentiate into dopaminergic neuron-like cells. *Stem Cells Dev*. 19(9):1375-83.
- Welte K, Platzer E, Lu L, Gabilove JL, Levi E, Mertelsmann R, and Moore MA. (1985) Purification and biochemical characterization of human pluripotent hematopoietic colony-stimulating factor. *Proc Natl Acad Sci U S A*. 82(5):1526-30.
- Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, Böhm M, Nickenig G. (2005) Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl J Med*. 353(10):999-1007.
- Westerweel PE, Luijten RK, Hoefler IE, Koomans HA, Derksen RH, Verhaar MC. (2007) Haematopoietic and endothelial progenitor cells are deficient in quiescent systemic lupus erythematosus. *Ann Rheum Dis*. 66(7):865-70.
- Willing AE, Vendrame M, Mallery J, Cassady CJ, Davis CD, Sanchez-Ramos J, Sanberg PR. (2003) Mobilized peripheral blood cells administered intravenously produce functional recovery in stroke. *Cell Transplant*. 12(4):449-54.
- Wojakowski W, Tendera M, Michalowska A, Majka M, Kucia M, Maslankiewicz K, Wyderka R, Ochala A, Ratajczak MZ. (2004) Mobilization of CD34/CXCR4+, CD34/CD117+, c-met+ stem cells, and mononuclear cells expressing early cardiac, muscle, and endothelial markers into peripheral blood in patients with acute myocardial infarction. *Circulation*. 110(20):3213-20.
- Wojakowski W, Tendera M, Zebzda A, Michalowska A, Majka M, Kucia M, Maslankiewicz K, Wyderka R, Król M, Ochala A, Kozakiewicz K, Ratajczak MZ. (2006) Mobilization of CD34(+), CD117(+), CXCR4(+), c-met(+) stem cells is correlated with left ventricular ejection fraction and plasma NT-proBNP levels in patients with acute myocardial infarction. *Eur Heart J*. 27(3):283-9.
- Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, Fichtner S, Korte T, Hornig B, Messinger D, Arseniev L, Hertenstein B, Ganser A, Drexler H. (2004) Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet*. 364(9429):141-8.
- Woodbury D, Schwarz EJ, Prockop DJ and Black IB. (2000) Adult rat and human bone marrow stromal cells differentiate into neurons. *J. Neurosci. Res*. 61, 364-370.
- Wright DE, Bowman EP, Wagers AJ, Butcher EC, Weissman IL. (2002) Hematopoietic stem cells are uniquely selective in their migratory response to chemokines. *J Exp Med*. 195(9):1145-54.
- Wurmser AE, Gage FH. (2002) Stem cells: cell fusion causes confusion. *Nature*. 416(6880):485-7.
- Yamamoto S, N Yamamoto, T Kitamura and M Nakafuku. (2001). Proliferation of parenchymal neural progenitors in response to injury in the adult rat spinal cord. *Exp Neurol* 172:1115-1127.
- Yoo SW, Kim SS, Lee SY, Lee HS, Kim HS, Lee YD, Suh-Kim H. (2008) Mesenchymal stem cells promote proliferation of endogenous neural stem cells and survival of newborn cells in a rat stroke model. *Exp Mol Med*. 40(4):387-97.

- Zachar V, Rasmussen JG, Fink T. (2011) Isolation and growth of adipose tissue-derived stem cells. *Methods Mol Biol.* 698:37-49.
- Zhang CP, Fu XB. (2008) Therapeutic potential of stem cells in skin repair and regeneration. *Chin J Traumatol.* 11(4):209-21.
- Zhang X, Hirai M, Cantero S, Ciubotariu R, Dobrila L, Hirsh A, Igura K, Satoh H, Yokomi I, Nishimura T, Yamaguchi S, Yoshimura K, Rubinstein P, Takahashi TA. (2011) Isolation and characterization of mesenchymal stem cells from human umbilical cord blood: reevaluation of critical factors for successful isolation and high ability to proliferate and differentiate to chondrocytes as compared to mesenchymal stem cells from bone marrow and adipose tissue. *J Cell Biochem.* 112(4):1206-18.
- Zhu J, Wang X, Chen J, Sun J, Zhang F. (2006) Reduced number and activity of circulating endothelial progenitor cells from patients with hyperhomocysteinemia. *Arch Med Res.* 37(4):484-9.
- Zohlnhöfer D, Dibra A, Koppa T, de Waha A, Ripa RS, Kastrup J, Valgimigli M, Schömig A, Kastrati A. (2008) Stem cell mobilization by granulocyte colony-stimulating factor for myocardial recovery after acute myocardial infarction: a meta-analysis. *J Am Coll Cardiol.* 51(15):1429-37.
- Zou Y, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. (1998) Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. *Nature* 393:595-599



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When most types of human tissue are damaged, they repair themselves by forming a scar - a mechanically strong 'patch' that restores structural integrity to the tissue without restoring physiological function. Much better, for a patient, would be like-for-like replacement of damaged tissue with something functionally equivalent: there is currently an intense international research effort focused on this goal. This timely book addresses key topics in tissue regeneration in a sequence of linked chapters, each written by world experts; understanding normal healing; sources of, and methods of using, stem cells; construction and use of scaffolds; and modelling and assessment of regeneration. The book is intended for an audience consisting of advanced students, and research and medical professionals.

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