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Bone marrow cells differentiation into organ cells using stem cell therapy

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Abstract. – Bone marrow cells (BMC) are progenitors of bone, cartilage, skeletal tissue, the hematopoiesis-supporting stroma and adipocyte cells. BMCs have the potential to differentiate into neural cells, cardiac myocytes, liver hepatocytes, chondrocytes, renal, corneal, blood, and myogenic cells. The bone marrow cell cultures from stromal and mesenchymal cells are called multipotent adult progenitor cells (MAPCs). MAPCs can differentiate into mesenchymal cells, visceral mesoderm, neuroectoderm and endoderm in vitro. It has been shown that the stem cells derived from bone marrow cells (BMCs) can regenerate cardiac myocytes after myocardial infarction (MI). Adult bone marrow mesenchymal stem cells have the ability to regenerate neural cells. Neural stem/progenitor cells (NS/PC) are ideal for treating central nervous system (CNS) diseases, such as Alzheimer's, Parkinson's and Huntington disease. However, there are important ethical issues about the therapeutic use of stem cells. Neurons, cardiac myocytes, hepatocytes, renal cells, blood cells, chondrocytes and adipocytes regeneration from BMCs are very important in disease control. It is known that limbal epithelial stem cells in the cornea can repair the eye sight and remove symptoms of blindness. Stem cell therapy (SCT) is progressing well in animal models, but the use of SCT in human remains to be explored further.

Key Words:
Bone marrow cells, Stromal cells, Mesenchymal cells, Multipotent adult progenitor cells, Stem cell therapy.

Introduction

Blood cells can turn into both central nervous system neurons as well as peripheral neurons[1]. Bone marrow cells can also turn into neuronal cells. Bone marrow stem cells can give rise to a variety of hematopoietic cells and repopulate blood through-out the life. It has been shown that in mice, which are incapable of developing myeloid lineage and lymphoid lineage cells, the transplanted adult bone marrow cells migrated into the brain and differentiated into neuronal cells and expressed neuron-specific antigens. Such results showed that bone marrow-derived cells can provide an alternative source for neurons in patients with neurodegenerative diseases and central nervous system injuries. In these cases, cell cultures should be prepared under sterilizing condition in order to obtain pure cell lines. Bone marrow stromal cells are progenitors of several cells and can be differentiated into other types of stem cells such as neural and myogenic cells. The prospects for the use of these cells in local and systemic transplantations and possibly in gene therapy have also been suggested[2].

Post-natal bone marrow is composed of two main systems in distinct lineages, (1) the hematopoietic tissue proper, and (2) the associated supporting stroma. Bone marrow is the only known constituent, in which two separate and distinct stem cells co-exist. Bone marrow cells form the hematopoietic microenvironment (HME), as these cells are the progenitor of stem cells of skeletal tissues. Recent research on stem cells suggested that bone marrow stromal cells have the potential to differentiate into of neurons, heart and myocytes. Bone marrow stem cells in a number of post-natal tissues display trans-germinal plasticity and ability to differentiate into various cell types. Therefore, bone marrow stromal cells have great therapeutic application for the treatment of various diseases and repair of damaged tissues or organs. For the *in vivo* or *ex vivo* manipulation of stem cells, it is very important to know the identity, nature, developmental origin and function of bone marrow stromal cells and their amenability.

Stem cells from bone marrow cultures co-purified with mesenchymal stem cells (MSCs) are called multipotent adult progenitor cells (MAPCs). MAPCs can differentiate into mesenchymal cells, visceral mesoderm, neuroecto-

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derm and endoderm characteristics *in vitro*. It was shown that when MAPCs were injected into an early blastocyst, they differentiated into different somatic cells. On transplantation into a non-irradiated host, MAPCs engraft and differentiate to the haematopoietic lineage, in addition to the epithelium of liver, lung and gut. Engraftment in the haematopoietic system and the gastrointestinal tract is increased when MAPCs are transplanted in a minimally irradiated host. MAPCs can proliferate without senescence or loss of differentiation potential; therefore, they are considered an ideal cell source for therapy of degenerative diseases.

Bone marrow cells are soft, sponge-like cells inside bones containing hematopoietic, blood-forming stem cells and are different from embryonic stem cells. Some cells are formed in the blood stream and called as peripheral blood stem cells (PBSCs). The umbilical cord blood (UCB) contains hematopoietic stem cells (HSC), which are used in transplantations. The bone marrow transplantation (BMT) and peripheral blood stem cell (PBSCT) transplantation are methods of stem cells to restore the function of destroyed cells by high doses of chemotherapy or radiation in cancer patients. BMT is frequently required for leukemia patients and some other diseases.

Bone Marrow Stem Cells and Cardiac Myocytes

Results obtained from prior studies[3] showed that the stem cells derived from bone marrow stem cells can regenerate myocardial infarcted cells after myocardial infarction (MI). Significant improvement of left ventricular function has been reported in patients with acute myocardial infarction. Bone marrow stem cells can also be differentiated into neurons, hepatocytes, cardiac myocytes and muscle cells[4-7]. Results obtained from clinical trials[8-11] suggested that stem cell therapy is safe and feasible

Bone marrow has a heterogeneous mix of cells, and different sets of bone marrow stem cells showed to have diverse direct or indirect effects. The mesenchymal stem cells and endothelial progenitor cells demonstrated the potential to regenerate cardiac myocytes through different mechanisms[12-14]. BMSCs can repair injured muscle and even express the markers of myogenic progenitors. These cells show no intrinsic myogenicity, but when co-cultured in a myogenic environment, a subset of $CD45^-$ bone marrow cells isolated from muscle adopts a myogenic cell fate. All these biological processes show a great therapeutic potential of bone marrow stem cells. Molecular biology and genetic methods with clinical research can help to define the mechanism and cell populations involved in these processes.

Adult stem cells undergo new patterns of development by a process known as trans-differentiation or plasticity. It has been suggested that cell-cell fusion as an alternate interpretation for transdifferentiation. Myocardial regeneration has been widely studied for stem cell therapy and plasticity. The cardiomyocytes were generated in animal models before and considered as a post-mitotic organ. The postmortem microscopic analysis suggested that a renewal achieved by stem cells that infiltrated normal and infarcted myocardium.

In order to achieve a good result in the repair of myocardial cells using in stem cell therapy, it is essential to develop sophisticated and advanced techniques and surgical methods. This can be achieved by tracking radio-labeled stem cells when they migrate into myocardial infarcted cells in the heart. Environmental and genetic factors are also very important in stem cell therapy.

Stamm et al[16] described the autologous bone marrow stem-cell transplantation for myocardial regeneration. Implantation of bone marrow stem cells near the heart muscle is a new method to restore tissue viability after myocardial infarction (MI).

It has been shown that the injection of autologous AC133+ bone-marrow stem cells to patients with myocardial infarctions produced good results. The implantation of AC133+ stem cells to the heart border zone induced angiogenesis and improved perfusion of the infarcted myocardium. In the majority of cases, the global left-ventricular function was enhanced, and infarct tissue perfusion improved remarkably. Strauer et al[17] studied the regeneration of human infarcted heart muscle by intracoronary autologous bone marrow cell transplantation in chronic coronary artery disease.

Bone Marrow Stem Cells and Neural Cells

The neural progenitor cells derived from adult bone marrow mesenchymal stem cells were found to promote neuronal regeneration[18]. The NS/PC, neural stem/progenitor cells are an ideal cell type for the treatment of diseases in central nervous system (CNS). But some ethical issues

has halted the use of fetal NS/PC as a source for stem cell therapy. The autologous bone marrow mesenchymal stem cells (BMMSCs) can trans-differentiate into neural progenitor cells (NPC). However, the biological function of BMMSC derived NPC (MDNPC) in neuronal systems is still unknown. It was shown that MDNPC can promote *in vitro* neural regeneration, which is a process comprising the generation of neurons and neurotransmitters. It was also shown that MDNPC and NS/PC had similar morphologies and no significant differences were detected between MDNPC and NS/PC in promoting PC12 cell proliferation, neurite outgrowth, and dopamine release.

NS/PC induced dopamine secretion via up-regulating the dopamine transporter (DAT). The MDNPC was comparable to NS/PC in promoting neural regeneration, which indicated that MDNPC is a promising source of neural stem cells for the treatment of neurological diseases. Anbari et al[19] studied the results of intravenous transplantation of bone marrow mesenchymal stem cells which promoted neural regeneration after traumatic brain injury. In Wistar rat model of brain injury, the supplement of lost nerve cells of traumatic brain injury was investigated by intravenous administration of allogeneic bone marrow mesenchymal stem cells. The traumatic brain injury was established by weight drop impact acceleration method and administration of rat bone marrow mesenchymal stem cells via a lateral tail vein. Results showed that 14 days after cell transplantation, the neurological function was significantly improved. Such findings indicated that intravenously administered bone marrow mesenchymal stem cells can promote nerve cell regeneration in the injured cerebral cortex, which supplements the lost nerve cells.

Bone marrow stem cells are characterized by self-renewal and their ability to become any type of cell which is required by the living organisms. Stem cells can be directed to differentiate into neurons or glial cells *in vitro*. The possible use of bone marrow stem cells in the treatment of illnesses such as Parkinson's disease, amyotrophic lateral sclerosis (ALS) and Alzheimer's disease is in the center of attention in many neuroscience research labs[20]. Stem-cell-based therapy has been used for the repair of spinal cord injury[21]. The spinal cord injury (SCI) results in loss of nerve tissue and loss of motor and sensory functions. Currently, there is no reliable treatment available for restoring the injury-induced loss of nervous function. The transplantation of stem cells or progenitors by stem cell therapy may be useful in repairing the damaged spinal cord. The neuroprotective and axon regeneration promoting effects have also been seen in transplanted stem cells.

Bone Marrow Stem Cells and Hepatocytes

Bone marrow stem cells can be regenerated into hepatocytes of liver. Grompe[(22)] described the role of bone marrow stem cells in the regeneration of the liver. Hepatic oval cells have been shown to be involved in liver regeneration. Bone marrow stem cells can give rise to several hepatic epithelial cell types, which include oval cells, hepatocytes and duct epithelium. Such observations show that bone marrow resident stem cells, specifically hematopoietic stem cells (HSC), can be considered as an important source for the replacement of damaged epithelial cells during chronic liver injury. Although fully functional bone marrow-derived hepatocytes do exist, they are rare and are generated by cell fusion, and not by stem cell differentiation. It has been suggested that bone marrow-derived stem cells may play an important indirect role in the regeneration of the liver. Hematopoietic cells, including lymphocytes, neutrophils, macrophages and platelets, can provide factors required for repairing the damaged liver tissue. In animal models, it was shown that hepatocytes and cholangiocytes can be derived from bone marrow stem cells. There have been studies reporting about the possible use bone marrow stem cells for treating the liver damages in humans[23]. Results obtained from that study showed that human hepatocytes and cholangiocytes could be derived from extrahepatic circulating stem cells and were probably of bone marrow origin, and such "trans-differentiation" could replenish large numbers of hepatic parenchymal cells.

Bone Marrow Stem Cells and Renal Cells

Prior studies showed that, in kidney injuries, bone marrow stem cells are involved in the healing process[24]. Medical researchers supported the existence of pathways, in rodents and humans that allow adult stem cells to be flexible in their differentiation repertoires. About 30 years ago, it was suggested that circulating endothelial precursor cells, derived from the bone marrow could contribute to the repair of renal vessels after transplantation[25]. The tissues or organs in adult

organisms contain a small population of cells which are capable of self-maintenance and can effectively proliferate and produce multipotent stem cells[26].

During the development of kidney, meta nephric mesenchymal stem cells can generate all types of epithelial cell other than those of the collecting ducts. It is not known whether such stem cells are present in adults[27]. In an autoradiographic analysis, normal adult Sprague-Dawley rats received either a single or repetitive injection of the DNA precursor 3H-thymidine (3H-TdR). Results obtained from that study showed that the majority of labeled cells observed in glomerular tufts were endothelial cells. Mesangial cells had a lower production rate. Podocytes showed no sign of proliferation. Bowman's capsule cells revealed a higher labeling index than tuft cells at all times[28]. The adult mammalian kidney cells have some repair capability. Tubular cells can divide, and tubules may regenerate after injury, and this process involves epithelial-mesenchymal transition (EMT). It seems that bone marrow can act as a renal stem cell compartment. Bone marrow has been considered as the third stem cell compartment for the liver (after hepatocytes and cholangiocytes)[29]. The mesenchymal stem cells isolated by adhesion to plastic can contribute to bone, cartilage, and cardiac muscle, but not to blood or liver, whereas hematopoietic stem cells isolated by cell sorting can contribute to liver, cardiac muscle and vasculature[30]. Papadimou et al[31] studied the direct reprogramming of bone marrow stromal cells into functional renal cells using cell-free extracts. Human bone marrow stromal cells (BMSCs) can be reprogrammed into renal proximal tubular-like epithelial cells using cell-free extracts. The human BMSCs form cross lineage boundaries toward renal cells via cell-extract reprogramming. The reprogrammed BMSCs acquire proximal tubular-like epithelial cell properties and integrate into proximal tubuli and protect from AKI in a mice model. It has been shown that Streptolysin-O-permeabilized BMSCs exposed to HK2-cell extracts undergo morphological changes and acquire epithelial functional properties such as trans epithelial-resistance, albumin-binding, and uptake and specific markers E-cadherin and aquaporin-1. Transmission Electron Microscopy (TEM) observations revealed the presence of brush border microvilli and tight intercellular contacts. It has been shown that EGFR pathway components are up-regulated in tubular epithelial cells.

The reprogrammed BMSCs can integrate into self-forming kidney tissue and form tubular structures. Use of reprogrammed BMSCs in immune deficient mice with cisplatin-induced acute kidney injury has been shown to improve kidney function. This is an evidence showing that reprogrammed BMSCs are a promising cell source for future stem cell therapy.

Bone Marrow Stem Cells and Chondrocytes

It has been established that mesenchymal progenitor cells are capable of repairing musculoskeletal tissue[32]. The study revealed a successful induction of *in vitro* chondrogenesis with human bone-marrow-derived osteochondral progenitor cells in a reliable and reproducible culture system. Human bone marrow was removed and fractionated, and adherent cell cultures were established. These cells were passaged into an aggregate culture system in a serum-free medium which contained type-I collagen (no type-II nor type-X collagen was detected). Type-II collagen was detected in the matrix by day 5, with the immune reactivity localized in the region of metachromatic staining. On day 4, type-II and type-X collagens were detected throughout the cell aggregates, except for the outer region of flattened, perichondrial-like cells in a matrix rich in type-I collagen. The aggrecan and link protein were detected in cell aggregates extracts, which suggested that the large aggregating proteoglycans found in cartilaginous tissues had been synthesized by the newly differentiating chondrocyte cells. The small proteoglycans, big lycan and decorin were also detected in these extracts. Immunohistochemical staining with antibodies specific for chondroitin 4-sulfate and keratan sulfate showed a uniform distribution of proteoglycans throughout the extracellular matrix of the cell aggregates. The bone-marrow-derived cell preparations were passaged in monolayer culture twice, and cells were allowed to grow to 100% confluence at each passage, and chondrogenic potential of cells was maintained after each passage. Tang and Wing[33] studied gene and stem cell-based therapies for cartilage regeneration and repair. They showed that the damaged or diseased articular cartilage was repaired by stem cell proliferation and chondrogenic differentiation. Several studies on bone marrow stem cell cultures have shown the regeneration of cartilage and chondrocyte cells. The mesenchymal stem cells are a group of clonogenic cells which are present in bone marrow stroma and are capable of multilin-

eage differentiation into mesoderm-type cells such as osteoblasts, adipocytes and chondrocytes[34]. Due to their isolation and differentiation potential, mesenchymal stem cells are an important tool in clinical medicine study and stem cell therapy.

Bone Marrow Stem Cells and Corneal Cells

The eye cornea provides us the vision as a window to see the world. Maintaining the corneal tissue transparency is essential for vision. The limbal epithelial stem cells in the cornea have been studied as a potential tool to restore the eye sight[35]. In the eye, the integrity and the ability of outermost corneal layer are very important. The epithelium plays an important role in the refraction of light on the retina at the back of the eye and in image creation. In the human eye, the cornea epithelium layer is maintained by stem cells, and this is a very interesting for those stem cell researchers looking for a way to repair the damaged human corneal cells. The potential of stem cells to regenerate corneal cells is very promising. However, it is very important to understand better how the stem cell therapy can be employed in the regeneration of ocular surface and to cure the blindness in humans.

The gene with the most significant effect on the development of stratified epithelia is p63. Ablation of the p63 gene in mice results in the absence of stratified epithelia. In human, mutations of the p63 gene cause disorders of the epithelia and of nonepithelial structures whose development depends on the epithelial functions. The p63 gene generates six isoforms[36]. The alternative splicing gives rise to 3 different C-termini, which are designated as α, β, and γ. In the ocular epithelium, the corneal stem cells contain only α isoform. The holoclones derived from the limbus are rich in α; the meroclones contain only a small quantity; the paraclones don't contain it. In normal resting corneal epithelium, no p63 isoform is present. Upon corneal wounding, cells originating from the limbus and containing α isoform migrate progressively through the epithelium of the peripheral and central cornea, and in the absence of an attached limbus, and no α isoform appears in the corneal epithelium. When migrating cells containing α isoform appear in the wounded corneal epithelium, they are confined to the basal layer, but the supra-basal cells contain β and γ mRNA. These data supported the concept that α isoform of p63 is necessary for the maintenance of the proliferative potential of limbal stem cells and their ability to migrate over the cornea. The β and γ isoforms are not stem cell markers, but are likely to play a role in epithelial differentiation during the process of corneal regeneration[36]. The therapeutic effects of topically applied BMSCs, bone marrow cells and CD117-positive hematopoietic stem cells (CD117+) on alkali-induced corneal ulcers have also been studied[37]. Results obtained from that study revealed that topical application of BMCs or CD117+ cells is an effective way to reconstruct corneal surface. The BMCs and CD117+ cells were integrated into the corneal epithelium.

Sanchez-Abarca Li et al[38] studied the human bone marrow stromal cells which differentiated into corneal tissue and prevented ocular graft-versus-host disease in mouse model. The clinical trials have shown the use of human bone marrow stromal cells (hBMSCs) for the treatment of immune-related disorders such as graft-versus-host disease (GVHD). This study showed that GFP+ transduced hBMSCs generated from bone marrow, migrated and differentiated into the corneal tissue of eye after subconjunctival injection in mice. The hBMSCs displayed morphological features of epithelial, stromal and endothelial cells and appeared at different layers and with different morphologies depending on their position within the epithelium. These cells displayed ultra-structural properties, such as bundles of intermediate filaments, inter digitations and desmosomes with GFP-negative cells, which confirmed their differentiation into corneal tissues. The GFP+ transduced hBMSCs were injected at different time-points into the right eye of lethally-irradiated mice undergoing bone marrow transplantation, which developed ocular GVHD (oGVHD). The hBMSCs massively migrated to corneal tissues after subconjunctival injection. Microscopic and histopathological examinations showed minimal or no evidence of GVHD in the right eye, while the left eye, where no hBMSCs were injected, displayed features of GVHD. These results showed that hBMSCs could induce their therapeutic effect by differentiation and regeneration of damaged eye tissues in the host. The hBMSCs represent a potential cellular therapy to attenuate ocular GVHD.

Bone Marrow Stem Cell Cultures and Media Preparations

The bone marrow is aspirated from the femoral shaft of the hip by puncturing the iliac crest under sterilized conditions according to eth-

ical standards[39]. The mononuclear cells (MNC) from bone marrow are isolated and cultured in cell culture medium. These aspirates are diluted 1:5 v/v with 2 mM EDTA/PBS (Merck, Darmstadt, Germany). The MNC-fraction is isolated by density gradient centrifugation at 435 g for 30 min at room temperature using Ficoll-Hypaque solution (Amersham, Freiburg, Germany), and seeded at a density of 10^6 cells/cm^2 into T75 or T175 cell culture flasks (Nunc, Wiesbaden, Germany). The first change of medium is performed within 3 days after isolation of bone marrow cells. The resulting fibroblastoid adherent cells are termed BM-FAC (BM-derived fibroblastoid adherent cells) and cultivated at 37°C at a humified atmosphere containing 5% CO_2. The expansion media either consisted of MSCGM(Mesenchymal Stem Cell Growth Medium Bullet Kit, Cambrex, Verviers, Belgium), or Dulbecco's modified Eagle's medium-low glucose containing 10% mesenchymal stem cell growth supplements (DMEM-lg and MSCGS; Cambrex). The fibroblastoid adherent cells are maintained in MSCGM or DMEM-lg + 10% MSCGS until 70% to 90% confluence. These cells are then harvested at subconfluence using Trypsin (www.PromoCell, Heidelberg, Germany), as 2nd passage and, after that, these cells are replaced at a mean density of $1.3 \pm 0.7 \times 10^3$/cm^2. The generations of single separated, fibroblastoid colonies termed as CFU-F (fibroblastoid colony forming units) are obtained by initially seeding the MNC at a low density (10^3 to 10^4 cells/cm^2). The CFU-F are selected and isolated either using Trypsin (PromoCell) or by scraping them off from the surface of the cell culture plate with the tip of a pipette. The other methods of isolations of umbilical cord blood cells and adipose tissues are also used for the preparations of cell cultures.

The methods of isolation, characterization, proliferation, differentiation, and transplantation of mammalian neural stem cells have been described[40]. The multipotent precursor cells are also known as neural stem cells, which proliferate during the development of CNS, and give rise to transiently dividing progenitor cells that differentiate into the cell types that compose the neurons of the adult brain. The neural stem cells are defined as having a self-renewal capability. These neural stem cells have been isolated from many mammalian species, such as rats, mice, pigs as well as humans.

Isolated bone marrow stromal cells were cultured after density fractionation. Figures 1A Figure and 1B illustrate the cells 48 hrs and 10-days after plating[41]. Adult human mesenchymal stem cells from bone marrow are multipotent cells which can replicate as undifferentiated cells and have the potential to differentiate to lineages of mesenchymal tissues which include bone marrow stroma, bone, cartilage, tendon, fat, and muscle.

The prolonged *ex vivo* culture of human bone marrow mesenchymal stem cells study showed the influence of their supportive activity toward NOD/SCID-repopulating cells and committed progenitor cells of B lymphoid and myeloid lineages[42]. The mesenchymal stem cells are non-hematopoietic cells which can differentiate into adipocytic, osteocytic and chondrocyte tissues.

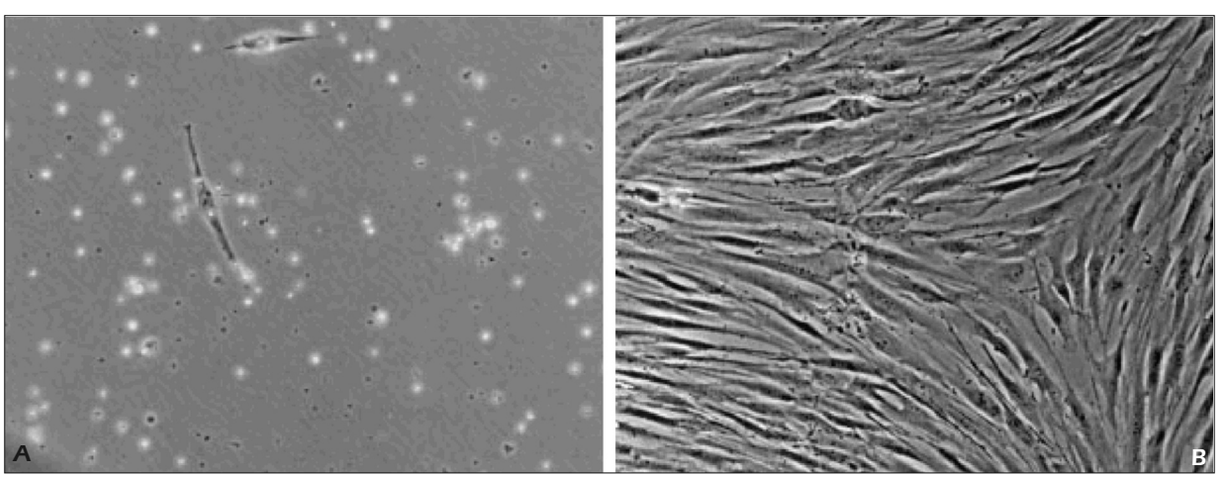

Figure 1. ***A,*** Adult human mesenchymal stem cells from bone marrow at 48 hrs of plating (adapted from Pittenger et al, 1999). ***B,*** Adult human mesenchymal stem cells from bone marrow at 10-days after plating (adapted from Pittenger et al, 1999).

A recent study[43] on the hematopoietic specification from human pluripotent stem cells discussed the existing challenges for *de novo* generation of the hematopoietic stem cells. Noteworthy achievements have been made in stem cell research and our understanding about the molecular and cellular pathways involved in stem cells differentiation has been expanded significantly. In recent years, we have been witnessing some important advances in bone regeneration techniques[44]. The clinical trials have shown the usefulness of adult stem cells, mesenchymal stem cells, endothelial progenitor cells, and $CD34^+$ blood progenitors for bone regeneration. Also, allogeneic bone scaffold enriched with concentrated autologous bone marrow cells obtained from the iliac crest, was shown to be a good alternative to treat acetabular bone defects observed in revision hip arthroplasty[45].

Conclusions

The bone marrow stem cells (BMSCs) have the ability to differentiate into blood cells and other types of cells in different organs, such as brain, spinal cord, heart, liver, kidney, bone, and cornea of the eye and adipocytes *in vitro*.

Stem cell therapy methods have been successfully used for repairing damaged tissues or organs in animals as well as humans. Although SCT has a great potential in treating several illnesses, we still have a long way before we can perfect the bone marrow transplantation techniques for stem cell therapies.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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