Introduction

Stem cells are key players in the development and maintenance of specific mammalian tissues, and their presence has been long established in blood, skin and intestine. A general definition of stem cell is “a cell that is capable of both self renewal and differentiation”. Stem cells are considered multipotent; since they can produces mature cell types of one or more lineages but cannot reconstitute the organism as a whole.

Continuous neurogenic process is sustained by the life-long persistence of neural stem cells (NSCs) within restricted CNS areas. In the adult mammalian brain, the genesis of new neurons has been consistently documented in the sub granular layer of the dentate gyrus of the hippocampus and the sub ventricular zone (SVZ) of the lateral ventricles. The SVZ is the adult brain region with the highest neurogenetic rate, from which NSCs have been first isolated and characterized for their ability to give rise to nonneural cells. Spinal cord injury (SCI) usually leads to devastating neurological deficits and disabilities (the annual incidence of SCI in the United States is estimated to be 40/million). Every year, over 20,000 cases of spinal cord injury patients are registered in our country.

Recently Eftekharpour et al explained cell replacement approaches in the recovery of SCI. Several other animal and human studies have demonstrated the tissue regeneration. These approaches indicate the
usage of neural stem cells and supportive cells in cell based therapy of SCI.

Though many sources of precursors cells have been applied in the recovery of spinal cord, the most convincing preclinical results have been obtained with (Neural progenitor cells) NPCs. However, these approaches have been most successful when applied in the sub acute phase of injury.

Some drugs, however, can lessen cord injury pathology but fail to influence the functional outcome. Also a few drugs are able to influence functional outcome without having any improvement on cord pathology. The goal of future treatment options for SCI is therefore to find suitable new drugs or a combination of existing drugs and to use various cellular transplants, neurotrophic factors, myelin-inhibiting factors, tissue engineering and nano-drug delivery to improve both the functional and the pathological outcome in the injured patient.

Classification of stem cells

Stem cells can be classified into two major categories, according to their developmental status: (i) Embryonic stem cells (ES) are pluripotent cells and capable of giving rise to most tissues of the organism. (ii) Adult stem cells are specialized cells found in many tissues of the body with their role in tissue homeostasis and repair.

Embryonic stem cells

Embryonic stem cells are pluripotent. They are able to differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm. Human ESCs are derived from discarded, non-transferred human embryos, from the inner cell mass of a blastocyst using an immunosurgical technique. Human neural stem cells / progenitor cells (derived from embryonic stem cells) differentiate into three neural lineages (neurons, astrocytes and oligodendrocytes) and are capable of forming mature progeny and also dopamine neurons in vitro or in vivo. The clinical application of such embryonic neural stem cells would be limited by the potential rejection from another individual’s immune system. To minimize this problem scientists developed individual’s somatic nucleus transfer technique (SCNT). Recently ESC derived neural stem cells identified by CD133 biomarkers were isolated by Fluorescence assay cell sorting (FACS). Compared to adult stem cells ES cells are clinically very effective for neurological disorders.

However, the ES cells also have some demerits such as in SCNT, not all of the donor cell’s genetic information is transferred, the resulting hybrid cells retain those mitochondrial structures which originally belonged to the egg. ES cells lines (>120 reported world wide) are gradually degraded and will soon be useless for research.

Adult stem cells: Adult stem cells are undifferentiated cells found throughout the body that divide to replenish dying cells and regenerate damaged tissues. They are found in higher number during embryonic development and less in adult. Adult stem cells have abilities to divide or self-renew. Unlike embryonic stem cells, the use of adult stem cells in research and therapy is not controversial because the production of adult stem cells does not require the destruction of an embryo. Adult stem cells can be isolated from a tissue sample obtained from an adult organ. They have mainly been studied in humans and model organisms such as mice and rats.

Neural stem cells: Self-renewing multipotent neural stem cells (NSCs) have been isolated and characterized from various areas such as the adult CNS including the spinal cord. Adult-derived neural progenitor and stem cells have been transplanted in animal models, and shown functional engraftment, supporting their potential use for therapy.

Site/origin of neural stem cells: In the mammalian adult brain, the genesis of new neurons continues throughout life within two 3-layered cortical regions, the hippocampus and olfactory bulb (OB), where it is sustained by endogenous stem cells. The most active NSC compartment is found in SVZ which represents a remnant of the embryonic germinal neuroepithelium, and persists throughout life as an active mitotic layer in the wall of the telencephalic lateral ventricles and along its rostral extension toward the olfactory bulb. A complete turnover of the resident proliferating cell population occurs every 12 to 28 days in the SVZ; about 30,000 new neuronal precursors (neuroblasts) being produced every day and migrating to the OB. Two main cell types are found in the SVZ: migratory, proliferating neuroblasts and astrocytes. These cells reach the more superficial OB layers and terminally differentiate into granule and periglomerular neurons. Glial tubes are composed of a special type of astroglia that expresses the marker of mature CNS astrocytes [glial fibrillary acidic protein (GFAP)] and also contain the cytoskeletal proteins vimentin and nestin.
Astroglial tubes and NSCs do not coexist solely within the periventricular aspect of the SVZ but also within the rostral migratory stream that extends into the OB, with the former perhaps contributing to create an appropriate stem cell “niche” for the maintenance of NSCs all along the pathway. In recent years, neurogenesis was reported to occur in other regions of the adult brain under normal conditions, such as neocortex, amygdala, and substantia nigra. However, other research groups were not able to replicate some of these reports.

The organization of the adult SVZ in humans is different from that in other mammalian species. The lateral ventricular wall consists of four layers with various thicknesses and cell densities: a monolayer of ependymal cells (layer I), a hypocellular gap containing astrocytic processes (layer II), a ribbon of cells composed of astrocytes (layer III), and a transitional zone into the brain parenchyma (layer IV). Astrocytes proliferate in vivo and behave as multipotent progenitors in vitro, but no chain migration was observed in the human SVZ. However, newborn cells that express cell cycle proteins [Ki-67 and proliferating cell nuclear antigen (PCNA)] were detected in the granular and glomerular layers of the human OB, but no clear evidence of the presence of a migratory pathway from the SVZ has been demonstrated. Therefore, it was suggested that individual cells might migrate separately to the OB. These results indicate that in comparison with rodents, precursor cells in the human OB are rare but not completely absent. However, these endogenous neural stem cells are very difficult to isolate in sufficient total number of cells from an individual patient’s brain tissue for immediate cell therapy.

Isolation and culturing of neural stem cells: The neural stem cells have been isolated and expanded from the embryonic and adult mouse striatum in the early 1990s in a culture system referred to as the neurosphere. Later it was found that not only embryonic CNS but also adult CNS possess the ability to generate neurospheres forming cells in vitro, including neural epithelial progenitor (NEP) cells, radial glial cells, SVZ cells, and ependymal cells, that clonigenically generate neurons, astrocytes and oligodendrocytes in vivo. Rao provided compelling evidence that after exposure to high concentrations of epidermal growth factor (EGF) mitogen, type C amplifying progenitors of the adult SVZ function as stem cells in vitro and from neurospheres. It was clearly indicated that transformed cells do not possess stem cell characteristic in vivo. Ependymal cells, astrocytes, oligodendrocyte precursors and neural progenitor cells can form neurospheres like aggregates that can be passaged for a limited time period. Most studies have shown that the neural stem cells derived from the brain respond to either basic fibroblast growth factor (bFGF) or EGF and neural stem cells cultured as neurospheres from the early embryonic forebrain do not respond to EGF until they acquire EGF receptors at later stages of development in vitro or in vivo. However, neural stem cell cultures from the adult murine hippocampus forms monolayer in the presence of bFGF.

These neurospheres produce repeated passages containing self renewing, proliferating and differentiating cells, typically presenting prominin -1 cell surface antigen which is also known as cluster of differentiation (CD) 133 and these cells are separated directly by magnetic beads conjugated with antibodies (MACS) or FACS by negative selection of CD 34 and CD 45: antigen markers cells (CD133+CD34+CD45-). Upon transplantation into brains of immunodeficient neonatal mice, the sorted /expanded CD133+ cells showed potent engraftment, proliferation, migration, and neural differentiation. Stem like cells have earlier been purified from various organs as side population (SP) cells, based on their property to exclude Hoechst.

Identification and characterization of neural stem cells

Immunophenotyping of isolated neural cells – Flowcytometer (FACS) analysis: Neural stem cells (NSCs) have been isolated from several regions of the brain from mice, rats, monkey and humans. These cells possess the characteristics of self-renewal and differentiation along all major neural lineages. Neural stem cells were identified as expressing CD133+/CD 34/CD45-. Schwartz et al demonstrated high levels of the neural stem cell markers like CD133, CD44, CD81, CD184, CD90, and CD29 positive but CD133 cell population was decreased in high-passage, lineage-restricted cultures. CD14 and CD144 were negative. Further Panchision et al identified distinct CNS precursor subtypes, including CD133, CD15, CD24, A2B5, and PSA-NCAM. Highest expression of CD133 or CD15 occurred in a CD24+ population that was enriched in neuronal progenitor cells. Multipotent cells expressed CD133 and CD15 at lower levels than neuronal progenitors cells. They concluded that CD133 and CD15 can be used as selectable markers, but CD24 coexpression helps to distinguish foetal mouse
multipotent stem cells from neuronal progenitors and postmitotic neurons. However, others investigated and identified neural stem cells by the expression of aldehyde dehydrogenase activity, SP cells (property to exclude Hoechst 33342), Syndecan -1, Notch -1 and integrin beta 1, CD 49d, Pruszak et al. sorted heterogeneous hESC-derived neural cell populations by FACS. Using genetically labeled synapsin-green fluorescent protein-positive hESC-derived neurons as a proof of principle, they enriched viable differentiated neurons by FACS. Cell profiling of surface antigens was identified for immature embryonic stem cell types such as stage-specific embryonic antigen [SSEA]-3, -4, TRA-1-81, TRA-1-60, neural stem and precursor cells such as CD133, SSEA-1 [CD15], A2B5, forebrain surface embryonic antigen-1, CD29, CD146, p75 [CD271], and differentiated neurons such as CD24 or neural cell adhesion molecule [NCAM; CD56]. NCAM (CD56) was used to isolate hESC-derived neurons (differentiated brain immature cells) by FACS. These heterogenous surface protein profile expression on neural development stages gives some more other immunophenotypic markers needed for the selection of markers for isolation, identification of neural stem cells in SVZ of foetal or adult brain.

Alternative sources of neural stem cells/progenitor cells for cell therapy

(i) Olfactory ensheathed cells (OECs) / Olfactory mucosa cells: The nose contains neurons that send signals to the brain when triggered by odour molecules. The axons of these neurons are enveloped by OECs, a special type of neuronal support cells (glial cells) that guide the axons and support their elongation. The bundles travel from the nose to the brain’s olfactory bulb, where these make connections with other neurons. Because olfactory tissue is exposed to the external environment (i.e., the air), it contains cells with considerable regeneration potential, including renewable neurons, progenitor/stem cells, and OECs. Through a relatively innocuous biopsy procedure, olfactory tissue can be obtained from the nasal cavity. It can also be retrieved from the olfactory bulb, but this requires an invasive penetration of the cranial cavity that although unsuitable for human patients, has been a procedure for most of the supporting animal research.

Problems of rejection, overgrowth, disease transmission and ethical issues can be avoided because a person’s own olfactory mucosa can be used. OECs theoretically promote axonal regeneration by producing insulating myelin sheaths around growing and damaged axons, secreting growth factors, and generating structural and matrix macromolecules that lay the tracks for axonal elongation.

These properties have led to an increased usage of olfactory ensheathing cells in preclinical models of transplantation for spinal cord repair including complete transection, hemisection, tract lesion, and contusion with over 50 published studies in the last 10 years.

Nasal olfactory ensheathing cells transplants assist recovery after spinal cord injury, including complete transaction and there is evidence that adult olfactory tissue is effective when transplanted one month after spinal cord transaction in the rat. According to the promising results obtained from animal experiments, several clinical trials have been started. In a large series more than 400 patients underwent transplantation of foetal olfactory bulb-derived cells, of which the results of 171 operations were published. A single-blinded, controlled trial has established the safety and feasibility of intraspinal transplantation of autologous olfactory ensheathing cells in human spinal cord injury. The safety and efficacy shown by researchers in implantation procedure are unclear. Patients have encountered serious medical complications and no lasting increase in sensory, motor function or functional ability. Recently electrophysiological evidence of olfactory cell transplants, improving the function of spinal cord injury is published.

When sampling of the transplanted OECs in to the spinal cord needs to be removed for transplantation studies from olfactory mucosa, it resulted in permanent damage to olfaction (smelling). Feron et al. tested the feasibility and safety of transplantation of autologous olfactory ensheathing cells into the injured spinal cord in human paraplegia. Olfactory ensheathing cells were grown and purified in vitro from nasal biopsies and injected by microinjection (12 to 28 million cells) into the region of damaged spinal cord. One year after cell implantation, there were no medical, surgical or other complications to indicate that the procedure was unsafe. There was no evidence of spinal cord damage or of cyst, syrinx or tumour formation. In the clinical examination, there was no neuropathic pain reported by the participants, no change in psychosocial status and no evidence of deterioration in neurological status. This indicated that the olfactory ensheathing...
cells transplantation may be a safe method by *in vitro* propagation before transplantation. In recent reports, others OECs cell transplantation studies have shown some adverse effects. Chen *et al.* reported that his sixteen patients experienced various complications including headache, short-term fever, seizure, central nerve system infection, pneumonia, respiratory failure, urinary tract infection, heart failure, and possible pulmonary embolism; there were 4 deaths. Chew *et al.* reported that a woman who received an injection into each frontal lobe in Beijing, China, progressed at a more rapid rate after the procedure and suffered disabling side-effects. In another clinical trial phase I/IIa was designed to test the feasibility and safety of transplantation of autologus olfactory ensheathing cells into the injured spinal cord in human paraplegia. There were no adverse findings 3 years after autologous transplantation of olfactory ensheathing cells into spinal cords injured at least 2 years prior to transplantation. The magnetic resonance images (MRIs) at 3 years showed no change from pre-operative MRIs or intervening MRIs at 1 and 2 years, with no evidence of any tumour of introduced cells and no development of post-traumatic syringomyelia or other adverse radiological findings. There were no significant functional changes in any patients and no neuropathic pain. They concluded that transplantation of autologous olfactory ensheathing cells into the injured spinal cord was feasible and safe up to 3 years of post-implantation; however, this conclusion should be considered preliminary because of the small number of trial patients.

(ii) Bone marrow (BM): The bone marrow stroma contains mesenchymal stem cells (also called marrow stromal cells). These cells are multipotent stem cells that can differentiate into a variety of cell types.

Recently the isolation and expansion of human Mesenchymal stem cells (MSCs) has been described. The potential of bone marrow cells to differentiate into myelin-forming cells and to repair the demyelinated rat spinal cord *in vivo* was studied using cell transplantation techniques. Sasaki *et al.* observed that the dorsal funiculus of the spinal cord was demyelinated with X-irradiation treatment, followed by microinjection of chidium bromide. Haematopoetic and non-haematopoetic stem cell, precursor cells, and lymphocytes were transplanted into the demyelinated dorsal column lesions of immunosuppressed rats. These genetically labelled bone marrow cells remylinated a peripheral pattern of myelination reminiscent of schwann cell myelination. However transplanted CD34 (+) haematopoietic stem cells survived in the lesion, but did not form myelin. These results indicate that bone marrow cells can differentiate *in vivo* into myelin-forming cells and repair demyelinated CNS. It is confirmed that non haematopoetic stem (CD 34) cells have capacity to differentiate into neural like cells. Such cells, when exposed to differentiation media, can develop electrophysiological characteristics of neurons, neuron-like MSC, however, lacked voltage-gated ion channels necessary for generation of action potentials. Hofstetter *et al.* delivered MSC into the injured spinal cord and observed possible effects on functional outcome in animals rendered paraplegic. However, MSC given 1 wk after injury showed better results compared to the immediate transplantation in the form of significant number of surviving cells and improvement in gait. Histology 5 wk after transplantation in spinal cord injury revealed that MSC were tightly associated with longitudinally arranged immature astrocytes and formed bundles bridging the epicenter of the injury. MSCs constitute an easily accessible, easily expandable source of cells that may prove useful in the establishment of spinal cord repair protocols. MSCs co-cultured or induced with foetal spinal cord-derived neurospheres cells stimulate the development of extensive processes. Such cells promote the regeneration of injured spinal cord by enhancing tissue repair of the lesion, leaving apparently smaller cavities than in controls. Although the number of grafted MSCs gradually decreased, some treated animals showed remarkable functional recovery.

To clarify whether the mouse BMSC can migrate into the lesion and differentiate into the CNS cells when transplanted into the mice subjected to focal cerebral infarct or spinal cord injury, Lee *et al.* studied the BMSC cells harvested from mice and characterized by flowcytometry. The cultured BMSC expressed low levels of CD45 and high levels of CD90 and Sca-1 on flow cytometry. A large number of grafted cells survived in the normal brain 4 wk after transplantation in rats, many of which have been located close to the transplanted sites. These expressed the neuronal marker including NeuN, MAP2, and doublecortin on fluorescent immunohistochemistry. However, when the BMSC were transplanted into the ipsilateral striatum of the mice subjected to middle cerebral artery occlusion, many of the grafted cells migrated into the corpus callosum and injured cortex, and NeuN like markers were expressed 4 wk after transplantation. Similar results were obtained.
in the transplantation in the mice with spinal cord injury. However, many of the transplanted BMSC expressed GFAP, an astrocytic protein, in injured spinal cord. Neuhuber et al. studied the effect of this form of therapy in chronically paraplegic Wistar rats due to severe spinal cord injury (SCI). The results showed a clear and progressive functional recovery of the animals treated with BMSC transplantation, compared to controls. Grafted BMSC survived into spinal cord tissue, forming cell bridges within the traumatic centromedullary cavity. In this tissue, cells expressing neuronal and astroglial markers appeared, together with a marked ependymal proliferation, showing nestin-positivity. These findings suggested the utility of BMSC transplantation in chronically established paraplegia. Lu et al. reported axonal regeneration beyond spinal cord injury sites achieved by combinatorial approaches (cAMP/NT-3) that stimulate both the neuronal soma and the axon, representing a major advance in strategies to enhance spinal cord repair. MSC transplants occupied the lesion cavity and were associated with preservation of host tissue and white matter (myelin), demonstrating that these cells exert neuroprotective effects. The tissue matrix formed by MSC grafts supported greater axonal growth than that found in specimens without grafts. Uniform random sampling of axon profiles revealed that the majority of neurites in MSC grafts were oriented with their long axis parallel to that of the spinal cord, suggesting longitudinally directed growth. Later demonstrated that BDNF-expressing marrow stromal cells support extensive axonal growth at sites of spinal cord injury.

Neuhuber et al. grafted human MSC derived from aspirates of four different donors into a subtotal cervical hemisection in adult female rats and found that cells integrated well into the injury site, with little migration away from the graft. Immuno cytochemical analysis demonstrated robust axonal growth through the grafts of animals treated with MSC, suggesting that MSC support axonal growth after spinal cord injury. However, the amount of axon growth at the graft site varied considerably between groups of animals treated with different MSC lots, suggesting that efficacy may be donor-dependent. Kamada et al. demonstrated that transplanted of Schwann cells derived from bone marrow stromal cells (BMSC-SCs) promotes axonal regeneration of lesioned spinal cord, resulting in recovery of hind limb function in rats.

Transplantation of bone marrow (BMT) cells into the injured spinal cord found to improve neurologic functions in experimental animal studies. However, it is unclear whether bone marrow cells can similarly improve the neurologic functions of complete spinal cord injury in human patients. To address this issue, Park et al. evaluated the therapeutic effects of autologous bone marrow cell transplantation (BMT) in conjunction with the administration of granulocyte macrophage-colony stimulating factor (GM-CSF) in six complete SCI patients. Sensory improvements were noted immediately after the operations. Significant motor improvements noted 3 to 7 months postoperatively. No immediate worsening of neurologic symptoms was found. Side effects of GM-CSF treatment such as fever (>38°C) and myalgia were noted. Serious complications increasing mortality and morbidity were not found. The follow-up study with magnetic resonance imaging 4-6 months after injury showed slight enhancement within the zone of BMT. BMT and GM-CSF administration represent a safe protocol to manage SCI patients, especially those with acute complete injury. It is yet to be confirmed that the observed beneficial effects are safe and worthy when unmanipulated autologous bone marrow cells are implanted in SCI patients. However, combined with macroporous polymer hydrogels based on derivatives of 2-hydroxyethyl methacrylate (HEMA) or 2-hydroxypropyl methacrylamide (HPMA) are suitable materials for bridging cavities after SCI. Li et al. suggested that inflammatory bone marrow-derived cells are the primary targets of A (2A) agonist-mediated protection. Recently, subarachnoid injection has been reported as a minimally invasive method of transplantation of bone marrow stromal cells for spinal cord injury. It may be, however, less effective than direct injection into the spinal cord in terms of cell delivery. Yoshihara et al. indicated that combining the beneficial effects of rat MSC and exercise protocol are not sufficient to enhance behavioural recovery. Cao et al. examined transdifferentiation of transplanted marrow stromal cells (MSCs) and reactive changes of glial cells in a completely transected rat spinal cord. Clinical studies are necessary for transferring preclinical findings from animal experiments to humans. Syková et al. investigated the transplantation of unmanipulated autologous bone marrow in patients with transversal spinal cord injury (SCI) with respect to safety, therapeutic time window, implantation strategy, method of administration, and functional improvement and a repeated data from 20 patients with complete SCI who received transplants 10 to 467 days post-injury. The implantation of autologous bone marrow cells appears to be safe, as there have been no complications.
following implantation to date (11 patients followed up for more than 2 years), but longer followups are required to determine that implantation is definitively safe. It is evident that transplantation within a therapeutic window of 3-4 wk following injury will play an important role in any type of stem cell SCI treatment. Trials involving a larger population of patients and different cell types are needed before further conclusions can be drawn. Cellular and extracellular inhibitors are thought to restrict axon growth after chronic spinal cord injury, confronting the axon with a combination of chronic astrocytosis and extracellular matrix-associated inhibitors that collectively constitute the chronic “scar.” Shi et al. showed MSCs enhanced angiogenesis in the rabbit host spinal cord and improved the motor functional recovery after spinal cord ischaemia. The therapeutic time window is critical for the therapeutic effect of MSCs. Koda et al. observed that numbers of double positive cells for GFP and glial markers were larger in the G-CSF treated mice than in the control mice after bone marrow cells of green fluorescent protein (GFP) transgenic mice were transplanted into lethally irradiated C57BL/6 mice. G-CSF showed efficacy for spinal cord injury treatment through mobilization of bone marrow-derived cells. Recently to assess the safety and therapeutic efficacy of autologous human bone marrow cell transplantation and the administration of GM-CSF, a phase I/II open-label and nonrandomized study was done on 35 complete spinal cord injury patients. BMCs were transplanted by injection into the surrounding area of the spinal cord injury site within 14 injury days (n = 17), between 14 days and 8 wk (n = 6), and at more than 8 wk (n = 12) after injury. In the control group, all patients (n = 13) were treated only with conventional decompression and fusion surgery without BMC transplantation. The patients underwent preoperative and followup neurological assessment, electrophysiological monitoring, and magnetic resonance imaging (MRI). The mean followup period was 10.4 months after injury. At 4 months, the MRI analysis showed the enlargement of spinal cords and the small enhancement of the cell implantation sites. BMC transplantation and GM-CSF administration were not associated with any serious adverse clinical events increasing morbidities.

We also transplanted autologous enriched mononuclear bone marrow stem cells (CD34) in spinal cord injury patients (unpublished data), and observed good results of clinical safety through open surgery transplantation.

(iii) Cord blood: Umbilical cord blood (UCB) is known to have stem/progenitor of cells. UCB is being used increasingly on an experimental basis as a source of stem cells (CD34+/45−), as an alternative to bone marrow. To date, more than 70 different diseases have been treated with cord blood transplants. Cord blood contains multiple populations of pluripotent stem cells, and can be considered as a best alternative to ES cells. Cord blood stem cells are capable of giving rise to haematopoietic, epithelial, endothelial and neural tissues both in vitro and in vivo. Thus, cord blood stem cells are amenable to treat a wide variety of diseases including cardiovascular, ophthalmic, orthopaedic, neurologic and endocrine diseases. Because of ethical issues the applications to human is prohibited in some countries. Saporta et al. tested whether human cord blood leukocytes ameliorate behavioral impairments of spinal cord injury and suggested that cord blood stem cells are beneficial in reversing the behavioral effects of spinal cord injury, even when infused 5 days after injury. Human cord blood-derived cells appeared in injured areas, but not in non injured areas, of rat spinal cords, and never appeared in corresponding areas of spinal cord of non injured animals. The results are consistent with the hypothesis that cord blood-derived stem cells migrate to and participate in the healing of neurological defects caused by traumatic assault. Intraspinal transplanted CD34+ CB cells achieved a better improvement in functional score than those that received BMS cells at days 7 and 14 after transplantation in rat model. Histological evaluation revealed that bromodeoxyuridine (BrdU)-labelled CB and BMS cells survived and migrated into the injured area. Some of these cells expressed glial fibrillary acidic protein (GFAP) or neuronal nuclear antigen (NeuN). Intraspinal transplantation of human CD34+ CB cells showed benefit in functional recovery after spinal cord hemisection in rats and suggested that CD34+ CB cells might be an excellent choice of cells as a routine starting material of allogenic and autologous transplantations for the treatment of spinal cord injury. Li et al. studied whether intraspinally transplanted human cord blood CD34+ cells can survive, differentiate, and improve neurological functional recovery after spinal cord injury in rats and he observed significant functional recovery in the group, which received human cord blood CD34+ cells compared with the control group. Transplanted hUCB
differentiated into various neural cells and led to motor function improvement in cord injured rat model. Nishio et al. suggested that transplanted CD34 positive fraction cells from hUCB may have therapeutic effects for SCI. These hematopoietic stem cells (CD 34+ cells) promoted restoration of spinal cord tissue and recovery of hind limb function in adult rats which provided important preclinical data regarding hUCB stem cell based therapy for SCI. Recently Dasari et al. investigated on axonal remyelination of injured spinal cord after hUCB cell transplantation. hUCB cells differentiated into several neural phenotypes including neurons, oligodendrocytes and astrocytes. Ultrastructural analysis of axons revealed that hUCB cells form morphologically normal appearing myelin sheaths around axons in the injured areas of spinal cord. These findings demonstrated that hUCB, when transplanted into the spinal cord 7 days after weight-drop injury, survived for at least 2 wk, differentiated into oligodendrocytes and neurons, and improved locomotor function. Due to difficulties in maintaining graft in the ageing rat CNS, Walczak et al. selected the NOD SCID mouse for in vivo characterization of human cord blood cells (hUCB). Stereotactically transplanted hUCB cells into the striatum and the brains have survived and differentiated into neuronal cells at either 5 or 30 days after transplantation. At early time points, many differentiated hUCB cells expressing characteristic neuronal proteins were detected. However, at 1 month post-grafting, hUCB cells were no longer detected. Recent findings in SCI rats treated with neurally induced progenitor cells (NPCs) of hUCB showed somatosensory-evoked potentials, and grafted cells especially exhibited oligodendrocytic phenotype around the necrotic cavity. Neurally induced progenitor cells of hUCB might be a therapeutic resource to repair damaged spinal cords. Many investigators demonstrated that CB stem cells are amenable to neurological applications including (as evidenced by in vitro studies) pre-clinical animal models of disease, and more recently by clinical trials. Therefore, umbilical CB stem cells are unique in their ability to be used for stem cell transplantation in the treatment of blood disorders, as well as use in regenerative medicine to treat patients with neurological disease.

(iv) Skin: The skin contains a precursor capable of generating neural cell types was indicated by the finding that Merkel cells, neural sensory receptors found in the dermis, can be generated in adult skin. Skin derived skin stem cells (SKPs) can generate both neural and mesodermal cell types and that most of the neural cells generated by SKPs have characteristics of peripheral neurons and Schwann cells, consistent with a potential neural crest origin. Recently a sub population of nestin–vimentin+ phenotype of fibroblasts cells appeared multipotent which showed neural cell differentiation characters.

Two independent research teams recently created induced pluripotent stem cells (iPS) from adult human cells. With the same principle used earlier in mouse models, Takahashi et al. had successfully transformed human fibroblasts into pluripotent stem cells using the same four pivotal genes: Oct3/4, Sox2, Klf4, and c-Myc with a retroviral system. Thomson and colleagues used OCT4, SOX2, NANOG, and a different gene LIN28 using a lentiviral system. Further, Park and team derived iPS cells (using Oct4, Sox2, Klf4 and Myc transcriptional factors) from foetal, neonatal and adult human primary cells, including dermal fibroblasts isolated from a skin biopsy of a healthy subject. They believed that these data demonstrated that, defined factors can reprogramme human cells to pluripotency, and can be established a method whereby patient-specific cells might establish in culture.

(v) Hair follicles: Hair follicles are known to contain a well-characterized niche for adult stem cells: the bulge, which contains epithelial and melanocytic stem cells. Nestin-positive cells are identified in the bulge area in mouse and can give rise to neurons, smooth muscle cells, and melanocytes. Neural-crest-like stem cells have been identified in mouse whisker hair follicles, and bulge cells from mouse whisker hairs grow as adherent monolayer cells and appear to be multipotent.

(vi) Adipose tissue: The adipose tissue is a highly complex tissue and consists of mature adipocytes, preadipocytes, fibroblasts, vascular smooth muscle cells, endothermal cells, resident monocytes/macrophages, and lymphocytes, hence this tissue compartment provides a rich source of pluripotent adipose tissue-derived stromal cells. Adipose tissue (AT) is another alternative source that can be obtained by a less invasive method and in larger quantities than BM. It has been demonstrated that AT contains stem cells similar to BM-MSCs, which are termed processed lipoaspirate (PLA) cells. These cells can be isolated from cosmetic liposuctions in large numbers and grown easily under standard tissue culture conditions. Processed lipoaspirated cells and clones differentiated into putative neurogenic cells, exhibiting a neuronal-like morphology and expressing several proteins consistent with the neuronal phenotype. The
multilineage differentiation capacity of PLA cells has been confirmed\textsuperscript{84}. Before application to human neurological diseases more in vitro experiments and preclinical trials are needed.

(vii) Wharton jelly: Wharton's jelly cells are isolated from the gelatinous connective tissue from the umbilical cord. The idea of Wharton's jelly as a source of primitive cell types, was based on the low levels of collagen expressed in gelatinous connective tissue and the fact that, during embryogenesis, totipotent cells, such as primordial germ cells and haematopoietic stem cells, migrate from the yolk sac through this region to populate target tissues in the embryo and foetus. Wharton's jelly cells possess one of the defining characteristics of stem cells, the ability to self-renew. Wharton's jelly cells have telomerase activity, which is found in human embryonic stem cells. Colonies of Wharton’s jelly cells also expressed NSE, c-kit, and TH, a marker for catecholaminergic neurons. Whether there is expression by the colonies of markers for non-neuronal cell lineages has yet to be determined\textsuperscript{85}

(viii) Amniotic placental fluid: Amniotic fluid contains a heterogeneous population of cells, which are contributed mainly from the foetal skin; the foetal digestive, respiratory, and urinary tract; and the placental membranes\textsuperscript{86-89}. Recent discoveries of stem cell populations in amniotic fluid have postulated that the amniotic fluid is a promising alternative source of fetal stem cells for cellular therapy\textsuperscript{90,95}.

(ix) Macrophages: Due to the immune privilege, recruitment of macrophages is limited in CNS and the resident microglia cells are the main immune cells that are activated after SCI\textsuperscript{86}. It has been shown that controlled boosting local immune response by delivering of autologous macrophages, can promote recovery from the spinal cord injury. Initial experiments with implantation of macrophages activated by preincubation with peripheral nerve fragments led to partial recovery of paraplegic rats\textsuperscript{97}. The postulated mechanisms are activation of infiltrating T cells, and increased production of trophic factors such as brain-derived neurotrophic factor (BDNF)\textsuperscript{98,99} leading to removal of inhibitory myelin debris. The results of phase I studies showed that out of eight patients in the study, three recovered clinically with significant neurological motor and sensory function. Also, it was shown that this cell therapy is well tolerated in patients with acute SCI\textsuperscript{100}.

(x) Dendritic cells: In animal model studies, transplantation of dendritic cells into the injured spinal cord of mice led to better functional recovery as compared to controls. The implanted dendritic cells induced proliferation of endogenous neural stem/progenitor cells (NSPCs) and led to de novo neurogenesis. This observation was attributed to the action of secreted neurotrophic factors such as neurotrophin-3, cell-attached plasma membrane molecules, and possible activation of microglia/macrophages by implanted dendritic cells\textsuperscript{101}. Dendritic cells pulsed (incubated) with encephalitogenic or nonencephalitogenic peptides derived from myelin basic protein when administered intravenously or locally to the site of injury, promoted recovery from SCI\textsuperscript{102}.

(xi) Schwann cells (SCs): Schwann cells are the supporting cells of the PNS. Like oligodendrocyte, Schwann cells wrap themselves around nerve axons, but the difference is that a single Schwann cell makes up a single segment of an axon’s myelin sheath. Schwann cells originating from dorsal and ventral roots are one of the cellular components that migrates to the site of tissue damage after spinal cord injury\textsuperscript{103-105}. The remyelinating capability of Schwann cells has been demonstrated in a number of studies\textsuperscript{104,106} and the functioning status of this myelin in conduction of neural impulses has confirmed\textsuperscript{107,108}.

(xii) Human foetus: Foetal derived multipotent foetal stem cells (FSCs) are generally more tissue-specific than ESCs. Therefore, FSCs are able to generate a more limited number of progenitor types. One of the particular therapeutic advantages of FSCs as compared to ESCs, is the fact that FSCs do not form teratomas in vivo. Moreover, the FSCs obtained up to week 12 offer the possibility of transplanting these primitive stem cells without frequent rejection reactions, in contrast to UCB and BM stem cell transplants. Recent work revealed the possibility of using FSCs or their progenitors, isolated from particular tissues, for multiple therapeutic applications involving tissue regeneration\textsuperscript{109-112}. FSCs can cross both the placental and blood-brain barrier resulting in improvement of therapeutic applications of neural stem cell progenitors in allowing their administration by iv infusion for repair of diverse brain disorders. Sub ventricular zone and hippocampus of foetal central nervous system contains more number of neural progenitor cells, can be directly isolated and transplanted. In our FACS data (unpublished) we found approximately 6 per cent of CD 56 (NCAM)/CD133\textsuperscript{+} profile immunophenotyped in MACS sorted human foetal neural CD 133 positive cells.


34. Lu J, Féron F, Ho SM, Mackay-Sim A, Waite PM. Transplantation of nasal olfactory tissue promotes partial


