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# Repairing neural injuries using human umbilical cord blood

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## Abstract

Stem cells are promising sources for repairing damaged neurons and glial cells in neural injuries and for replacing dead cells in neurodegenerative diseases. An essential step for stem cell based therapy is to generate large quantities of stem cells and develop reliable culture conditions to direct efficient differentiation of specific neuronal and glial subtypes. The human umbilical cord (hUC) and umbilical cord blood (UCB) are rich sources of multiple stem cells, including hematopoietic stem cells, mesenchymal stem cells, unrestricted somatic stem cells and embryonic-like stem cells. Human UC/UCB-derived cells are able to give rise to multiple cell types of neural lineages. Studies have shown that UCB and UCB-derived cells can survive in injured sites in animal models of ischemic brain damage and spinal cord injuries, and promote survival and prevent cell death of local neurons and glia. Human UCB is easy to harvest and purify. Moreover, unlike embryonic stem cells, the use of human UCB is not limited by ethical quandaries. Therefore, human UCB is an attractive source of stem cells for repairing neural injuries.

### Keywords

human umbilical cord blood; mesenchymal stem cells; neural stem cells; embryonic stem cells; neural injuries; neural repair

Stem cells are a population of cells capable of self-renewal and with the ability to generate multiple tissue types. Stem cells can be classified into totipotent, pluripotent and multipotent stem cells, based on their ability to differentiate. Totipotent stem cells only exist in early embryos, which differentiate into embryonic and extraembryonic cell types. Pluripotent stem cells are found in the inner cell mass of mammalian embryos, which have unlimited self-renewal properties and give rise to nearly all cell types, such as embryonic stem cells (ESCs). Multipotent stem cells can differentiate into a number of cells that are in a closely related family. For example, hematopoietic stem cells (HSCs), the well studied adult stem cells, have self-renewal properties and can give rise to a wide range of progenitors and mature cells largely within the confine of the hematopoietic system [1]. Mesenchymal stem cells (MSCs) are multipotent non-hematopoietic cells with the capacity to differentiate into osteoblasts, chondrocytes, adipocytes, as well as myogenic and neuronal cells [2, 3]. Moreover, neural stem cells (NSCs) are multipotent cells in the nervous system, which give rise to neurons, astrocytes and oligodendrocytes [4, 5].

Stem cells provide an alternative approach to therapy of various diseases for example neurological disorders and neurodegenerative diseases. The ethical issues of using ESCs and restricted sources of harvesting HSCs, MSCs and NSCs have limited the potential use of

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stem cells for therapy. Human umbilical cord blood (hUCB), which remains in the umbilical cord and placenta after birth, had long been discarded as wastes. However, the first successful cord blood transplantation to treat a patient with Fanconi Anaemia has drawn attention to use UCB as a source of hematopoietic stem cells for therapy [6]. In 2004, non-hematopoietic stem cells were isolated from the cord blood, which initiates the use of hUCB for regenerative medicine [7]. Since then, the human umbilical cord (hUC) and hUCB have been found to give rise to many types of stem cells such as HSCs, MSCs and NSCs. Due to the ease of access and collection as well as its ethical acceptance, UCB is increasingly becoming a reliable source of stem cells for therapy of many neural injuries and neurodegenerative diseases. In this review, we will emphasize neural subtypes derived from hUC and hUCB, and highlight therapeutic mechanisms of using hUC and hUCB for the treatment of neural injuries and neurodegenerative diseases.

## Stem cells from human umbilical cord blood and umbilical cord

Human UCB collected from the umbilical vein shortly after birth contains multiple types of stem cells (Table 1). Initially, human UCB was collected to isolate HSCs [6]. The population of HSCs in human UCB can be expanded and enriched *in vitro*, using CD34 as a selection marker. Compared to other sources of HSCs, UCB-derived HSCs are enriched with primitive stem/progenitor cells capable to produce *in vivo* long-term repopulating stem cells. A single unit of UCB contained enough hematopoietic stem cells to reconstitute definitely the host lympho-hematopoietic compartment [8]. Thus, UCB-derived HSCs can be used as a reliable source of HSCs for transplantation.

UCB also contains a population of MSCs. MSCs express the surface markers CD10, CD13, CD29, CD44, CD90 and CD105 but lack expression of CD31, CD34 and CD45 [9]. Compared to isolation from bone marrow (BM), isolation and expansion of MSCs from UCB is much easier. Moreover, hUC-MSCs exhibit greater proliferative activity than BM-MSCs [10]. Thus, UCB is a useful source for developing therapeutic strategies using MSCs.

A third stem cell population in UCB is called unrestricted somatic stem cells (USSCs). These cells express CD13, CD29, CD44, CD49e, CD90 and CD105 [7]. They possess the ability to differentiate into a wide range of cell types in the mesodermal, endodermal and ectodermal lineages [7]. Although the mechanisms underlying their multipotency are unclear, USSCs provide a useful source for stem cell transplantation.

Finally, UCB contains cord blood-derived embryonic-like stem cells (CBESs). CBESs form embryoid body-like colonies in culture and express markers such as CD133, CD164, Oct4, SSEA-3 and SSEA-4 [11, 12]. These features suggest that CBESs are similar to ESCs and can be used to produce many distinct cell types for potential stem cell-based therapy.

In addition to the stem cell populations in the cord blood, human umbilical cord itself contains stem cells. hUC is covered by elastic matrix of connective tissue called Wharton's jelly, which is comprised of mucopolysaccharides, macrophages and fibroblasts. Wharton's jelly is a rich source of MSCs, which can be mechanically or enzymatically collected [13]. Umbilical cord-derived MSCs, like other MSCs, can give rise to multiple cells such as endothelial cells, hepatocytes and neural lineages, making the umbilical cord also an ideal source of stem cells.

Human UCB is highly heterogeneous and is comprised of various stem cell populations that can give rise to cells in many different tissues [14]. Although several stem cell types have been identified, primarily through the use of cell surface markers, other unidentified cell types may be contained in UCB. Compared to other types of stem cells, human UC/UCB-derived stem cells have many advantages: 1) They are easily harvested and manipulated

without harm to the baby or mother; 2) They have abundant sources considering about 135 million births worldwide each year; 3) There are less ethical issues, as human UC/UCB has been taken as wastes for a long time; 4) They exhibit low immunogenicity in clinical applications; 5) They are associated with a lower risk of viral contamination. With the advantage of these features of human UCB and UCB-derived cells, UCB has been used as a rich stem cell pool for therapies in many human diseases and injuries, such as cardiac regeneration, vascular disorders, inherited metabolic disorders, diabetes and neurological disorders [15, 16].

## Generating neural cells from umbilical cord blood

Human UCB contains cells that can give rise to ESCs, MSCs and NSCs, which have capacity to differentiate into neural lineage including neurons, astrocytes and oligodendrocytes. We here summarize methods of generating NSCs, neural progenitors, neuronal and glial cells from human UCB.

### NSCs and neural progenitors

Embryonic-like stem cells are purified from the heterogeneous stem cell population in human UCB [12]. These stem cells are selected by the cell surface markers $CD45^-$, $CD33^-$, $CD7^-$ and $CD235a^-$ and also express embryonic stem cell markers such as Oct4 and Sox2. Due to their characteristic similarities to ESCs, embryonic-like stem cells can be directed into neural progenitors by adding morphogens such as retinoic acid (RA) and brain-derived neurotrophic factor (BDNF) [12].

Human UCB has been cultured and directly programmed into NSCs. UCB, which is negative for CD34 and CD45, was cultured for 6 weeks and subsequently treated with mitogens such as epidermal growth factor (EGF) [17]. Cells were then cultured at low density where they form clones that express the NSC marker nestin. These cells have the ability to form neurospheres and to differentiate into all neuronal cell types and glia [18, 19]. Neurons generated from UCB not only express neuronal markers but also exhibit electrophysiological properties characteristic of neurons [18].

### Neuronal subtypes

MSCs are usually cultured from mononuclear cells spun down from UCB [20, 21]. They are isolated using various methods, including selection in the culture medium by positive expression of surface markers such as CD13, CD29 and CD105 and negative expression of CD34, CD45, CD11b, CD3 and CD19 [3, 20]. Although neurogenic medium containing RA and basic fibroblast growth factor (FGF) has been reported to promote generation of neurons and glia directly from MSCs, one study has suggested that this medium has minor effects on the MSC transition to cells of neural lineages [22]. Conversely, both BDNF and nerve growth factor (NGF) have been found to induce neuronal differentiation of UCB through different signaling pathways [23, 24].

A careful study was performed to test the effect of neurotrophin and growth factors on the efficacy in neuronal induction of mononuclear UCB. Chen et al. found that the combination of neurotrophin and growth factors such as hSHH, FGF, EGF and RA promotes neuronal differentiation but not astrocyte production from mononuclear UCB [25]. Such combinational treatment also enhances the maturation of differentiated neurons. Human UCB cells survive longer after transplantation with the treatment of the combination of neurotrophin and growth factors [25]. Moreover, interferon-gamma has been found to enhance neuronal differentiation of UCB-derived cells *in vitro* in a does-dependent manner [26].

Extracellular matrix (ECM) has been shown to promote proliferation and neuronal differentiation of human UCB derived NSCs [27]. Matrix metalloproteinases (MMPs) play a role in modification of ECM and alter the ECM substrate interaction. MMPs were detected in human UCB derived NSCs (hUCB-NSCs). Inhibition of endogenous MMP activity in hUCB-NSCs that are cultured on ECM substrates significantly reduces their proliferation and differentiation towards the neuronal lineage [27]. Both fibronectin and collagen have been found to enhance proliferation of hUCB-NSCs [27]. These studies indicate an important role of extracellular matrix in neuronal induction from human UCB.

Furthermore, human UCB can be differentiated into specific neuronal types. After CD45 negative selection, UCB cells were cultured in media containing EGF and FGF for 10 days [28]. These cells express neural progenitor markers Pax6 and Tbr2. UCB cells were then cultured for 7 days with the addition of B27 and N2. Finally, BDNF, NGF and cyclic AMP were added to the medium to induce neuronal maturation. At this stage, Tbr1 expression was detected in UCB derived neurons. Subsequent expression of Pax6, Tbr2 and Tbr1 in UCB derived neurons is consistent with expression patterns observed in developing human and mouse cortices [28]. This study suggests that the defined culture conditions can direct human UCB into neural progenitors and postmitotic neurons, which recapitulates *in vivo* developmental stages in the human cortex.

## Glial cells

Human UCB can also differentiate into glial cells. Buzanska et al. demonstrated that in presence of RA or BDNF, CD34 and CD45 negative UCB-derived cells can give rise to about 30% neurons, 40% astrocytes and 11% oligodendrocytes [17]. UCB-derived mononuclear fraction cells have been shown to differentiate into neurons and astrocytes when induced by addition of RA and NGF into the culture medium. When the induced UCB-derived cells were transplanted into the anterior subventricular zone of neonatal rat brains, they showed immunopositive for neuronal and astrocyte markers, suggesting that UCB-derived cells can differentiate into both neurons and astrocytes *in vivo* [29]. On the other hand, transplanted UCB-derived cells without pretreatment with RA and NGF before transplantation showed no expression of markers for neurons, astrocytes and oligodendrocytes [30]. Moreover, a subset of mononuclear fraction of UCB, which express CD13, CD29 and CD44, but not antigens of hematopoietic differentiation, are positive for markers of neurons and astrocytes in the presence of EGF and FGF [31]. UCB-derived HSCs, which are selected by CD133, express markers of neurons, astrocytes and oligodendrocytes with the treatment of RA [32]. In addition, laminin has been shown to promote differentiation of hUCB-NSCs towards oligodendrocytes [33]. These studies indicate that neuronal and glial differentiation of hUCB-derived cells can be induced by neurotrophin and growth factors.

Taken together, these studies indicate that human UCB contains cells with a high plasticity, and can give rise to ESCs, MSC, NSCs or directly neurons and glial cells under optimal culture conditions. However, previous studies have shown that UCB can fuse with hepatocytes and cardiomyocytes [34, 35]. Therefore, when transplanted into animals, whether UCB directly differentiates into functional neurons or fuses with endogenous neurons remains further investigation. Moreover, reports have shown that the transplanted human UCB is either undifferentiated or is differentiated into nonfunctional neurons [36, 37]. Nevertheless, the high plasticity of human UCB makes it an ideal source for generating various neurons and glial cells.

## Umbilical cord blood for neural repairs

In recent years, usage of human UCB for repairing neural injuries has increased [38, 39] (Table 2). We here summarize treatment of neural injuries and neurodegeneration diseases using hUCB and UCB-derived cells mostly in rodent models.

### Ischemia

In mouse models of ischemia, transplanted UCB cells were found in injured sites in the cortex or striatum, suggesting the migration capability of transplanted UCB towards injured sites [40, 41]. Hepatocyte growth factor (HGF) appears play an important role in the migration of UCB, since blocking HGF function with a neutralizing antibody has been found to inhibit migration of UCB to the injured sites [37, 40]. In addition, increased levels of cytokines and chemokines such as cytokine-induced neutrophil chemoattractant-1 and monocyte chemoattractant protein-1 have been found in the ischemic tissue extracts with the migrated UCB cells, suggesting that they may function on the migration of UCB to the injured cortex and striatum after stroke [41].

Transplantation of human UCB or UCB derived cells has been proven to improve neurological function in ischemic models. In middle cerebral artery occlusion rat models, intravenous administration of human UCB or UCB derived NSCs reduces the infract volume and significantly improves neurological performance [42, 43]. The transplanted UCB cells have been found to transdifferentiate into neurons, astrocytes and endothelial cells. Intravenous injection of UCB has been found to be more effective than direct striatal implantation in producing long-term functional restoration to the stroke animals [44]. Moreover, the therapeutic window of stroke is usually limited within 3 hours in anticoagulant treatment in clinics. A longer therapeutic window, which extends to 24–72 hours after stoke event, was observed after transplantation of UCB, which may provide clinical benefits for the treatment of patients who have missed the 3 hours window [41].

Human UCB and UCB-derived cells play a critical role in neural protection and anti-inflammation in ischemic models. Intracranial injection of undifferentiated human UCB derived MSCs two weeks after middle cerebral artery occlusion has been found to improve neurobehavioral function and reduce the infarct volume. These MSCs express neuronal markers but are electrophysiologically inactive for neuronal type channels [36]. Granulocyte colony stimulating factor (G-CSF), vascular endothelial growth factor (VEGF), glial cell line-derived neurotrophic factor (GDNF) and BDNF were detected in differentiated and undifferentiated MSCs. The simultaneous injection of a BDNF-neutralizing antibody has been found to partially block the neurobehavioral amelioration, suggesting that the neuroprotective effect of BDNF rather than the integration of transplanted cells into the host cell networks plays a critical role in the recovery of neurobehavioral function [36]. In addition, intravenous injection of human UCB into stroke animal at 3 days after stroke together with the treatment with mannitol, which is the brain-blood-barrier (BBB) permeabilizer, has been found to promote the behavioral recovery and reduce the cerebral infarct volume. The levels of GDNF are increased significantly in both the brain and the blood after the UCB injection, which further suggests a neuroprotective role of transplanted UCB through the release of growth factors [45].

Moreover, microglia has been found to play a role in inducing neuronal damage in neurological injuries and neurodegenerative diseases [46]. UCB has been found to decrease microglial survival *in vitro* [47]. In neonatal hypoxic-ischemic brain damage rat models, UCB transplantation resulted in better performance in sensorimotor reflex tests, a decrease in the number of activated microglial cells and elevated levels of NGF and BDNF, further indicating a neuroprotective role of UCB [48-51]. The proinflammatory cytokines such as

TNF-α and IL-1β were found reduced, while the production of IL-10 increased, suggesting an anti-inflammation function of UCB transplantation [52-54].

UCB contains a high number of endothelial progenitor cells (EPCs) [55, 56]. UCB-derived cells such as mononuclear cells or MSCs are also able to differentiate into EPCs, which can induce angiogenesis, an important healing process that promotes neurogenesis in ischemic tissues [57-60]. The transplanted hUCB cells have been shown to induce neovascularization and angiogenesis [39, 61]. Therefore, UCB transplantation can enhance functional recovery through promoting angiogenesis in ischemic animal models [37, 62-64].

Taken together, UCB transplantation promotes functional improvement in animal models of ischemia through multiple mechanisms including neuroportection, anti-inflammation, angiogenesis and cell replacement. A clinical trial (NCT01700166) is designed to use human UCB to treat children with perinatal arterial ischemic stroke. Thus, human UCB is a promising stem cell source for the treatment of ischemia in humans.

## Spinal cord injuries

When human UCB is transplanted to the site of the injured spinal cord in the adult rodents, a significant recovery of locomotion was observed [65]. The transplanted UCB exhibited the ability to differentiate into neurons, astrocytes and oligodendrocytes [66]. Moreover, normal myelin sheaths around axons in the injured areas of the spinal cord is observed after UCB transplantation, suggesting that human UCB either directly participates or stimulates regeneration of damaged axons [37]. However, the transplanted UCB in the injured spinal cord doesn't display functional neuronal differentiation [63, 67]. On the other hand, both GDNF and VEGF were detected in the injured spinal cord, and the serum IL-10 levels were increased, but the TNF-α level was decreased after the transplantation of UCB [68]. These results support that anti-inflammation and neuroprotection induced by growth factors are involved in the beneficial effect of UCB transplantation in the spinal cord injury. Furthermore, downregulation of Fas and caspases was observed in injured areas, suggesting that UCB prevents cell death after spinal cord injuries [69]. Promisingly, a clinical trial (NCT01046786) that uses human UCB mononuclear cells for the treatment of spinal cord injuries is in phase II. Therefore, UCB may have a great potential for promoting recovery of spinal cord injuries.

## Neurodegenerative diseases

Human UCB and the umbilical cord also have a potential for the treatment of neurodegenerative diseases such as Parkinson's disease. Human UCB derived multipotent stem cells were induced to differentiate into tyrosine hydroxylase (TH) expressing dopaminergic neurons in the presence of all-trans-retinoic acid [70]. MSCs isolated from the Wharton's jelly of the umbilical cord were cultured to differentiate into dopaminergic neurons [71]. These neurons were transplanted into the striatum of a Parkinson's disease rat model. Migration of TH expressing neurons was detected in the striatum, suggesting survival of transplanted neurons.

In an Alzheimer's disease (AD) transgenic mouse model that expresses human amyloid precursor protein Swedish mutation, transplantation of human UCB has been found to prolong mouse survival [72]. A decline of amyloid-β plaques and a reduction of levels of soluble and insoluble amyloid-β were detected after transplantation of UCB [73]. A decrease of glial activation, oxidative stress and apoptosis was observed, and cognitive impairment was rescued in AD mouse models after transplantation of UCB derived MSCs. These studies indicate a therapeutic potential of UCB in neurodegenerative disorders such as AD. A phase I clinical trial (NCT01297218) of using human UCB-derived MSCs for the treatment of AD

patients is completed. Another clinical trial (NCT01696591) is designed to investigate the long-term safety of transplanted human UCB-derived MSCs in AD patients.

Moreover, human UCB was delivered intravenously into a mouse model of amyotrophic lateral sclerosis (ALS), a neurodegenerative disease that primarily affects motor neurons [74, 75]. Delayed disease progression and increased lifespan were observed in UCB treated diseased mice. A clinical trial (NCT01494480) of transplantation of UCB-derived MSCs in ALS patients is in phase II.

Therefore, human UCB has shown a great potential for treatment of neural injuries and neurodegenerative diseases. UCB and UCB-derived cells can survive upon transplantation at the damaged site and may directly differentiate into specific neuronal or glial cell types. UCB likely also plays a protective role by secreting neurotrophic factors. Human UCB exhibits their therapeutic potential through multiple mechanisms such as neuroprotection, anti-inflammation, anti-apoptosis as well as differentiation into neurons and glia (Table 2).

## Perspectives

Human UC and UCB are easy to harvest and purify. The highly heterogeneous UCB has a great potential to generate multiple stem cell types, including MSCs and NSCs, as well as specific neurons and glial cells. However, the heterogeneity of UCB has also created challenges for developing optimized culture conditions to maximally purify specific cell types from UCB. Moreover, molecular mechanisms that maintain pluripotency of UCB and direct UCB into neural lineages are still unclear. The specific molecular and functional features of UCB derived neurons remain to be explored. Whether UCB derived neurons can form functional connections after transplantation is unknown. To address these questions, thorough investigations at molecular and cellular levels will promote the research progress in this field, which is largely not yet ready for prime time. In addition, comprehensive studies of neurotrophic factors secreted from UCB will reveal the underlying mechanisms of neuroprotection by transplanted UCB in neural injuries. Fresh or cryopreserved UCB are undoubtedly good candidates for stem cell-based therapy and have a great potential in clinical application.

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## Table 1

Types of stem cells in human umbilical cord blood (UCB).

| Types of stem cells in UCB | Markers | References |
|---|---|---|
| Hematopoietic Stem Cells (HSCs) | $CD34^+$ | [6], [8] |
| Mesenchymal Stem Cells (MSCs) | $CD10^+$, $CD13^+$, $CD29^+$, $CD44^+$, $CD90^+$, CD31-, CD34-, CD45- | [9] |
| Unrestricted Somatic Stem Cells (USSCs) | $CD13^+$, $CD29^+$, $CD44^+$, $CD49e^+$, $CD90^+$, $CD105^+$ | [7] |
| Cord blood-derived Embryonic Stem Cells (CBES) | $CD133^+$, $CD164^+$, $Oct4^+$, $SSEA\text{-}3^+$, $SSEA\text{-}4^+$ | [11, 12] |

**Table 2**

Treatment of neurological disorders using human umbilical cord blood (UCB).

| Neurological disorders | Effects after transplantation of UCB and UCB derived cells | Working mechanisms | Species | References | Clinical trial No. |
|---|---|---|---|---|---|
| Ischemia | Reduce the infract volume; Improve neurological performance | Migrate to the injured sites; Cell replacement; Angiogenesis; Growth factors mediated neuroprotection; Anti-apoptosis; Anti-inflammation | Mouse, rat | [36], [39-45], [48-64] | NCT01700166 |
| Spinal Cord Injury | Promote regeneration; Recover locomotion | Migrate to the injured sites; Cell replacement; Growth factors mediated neuroprotection; Anti-apoptosis; Anti-inflammation | Rat | [37], [65-69] | NCT01046786 |
| Parkinson's Disease | No reports *in vivo* | Differentiate into dopaminergic neurons; Cell replacement | Rat | [70, 71] | |
| Alzheimer Disease | Prolong survival; Rescue cognitive impairment | Anti-inflammation; Anti-apoptosis; Reduce the levels of amyloid-β | Mouse | [72, 73] | NCT01297218, NCT01696591 |
| Amyotrophic Lateral Sclerosis | Delay disease progression; Increase lifespan | Cell replacement; Neuroprotection | Mouse | [74,75] | NCT01494480 |