

**AMELIORATIVE POTENTIALS OF STEM CELLS FROM HUMAN UMBILICAL CORD:  
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**ABSTRACT**

Stem cells have the potential to treat an enormous range of diseases and conditions that plague millions of people around the world. MSCs originate in the human embryo are considered multipotent stem cells. UC is considered as medical waste and the collection is noninvasive; has not been encumbered with ethical problems. There is no pain or risk for the mother or baby in extracting the blood from the umbilical cord, and the collection process is easily performed at the same time. Stem cell research has the potential to treat birth defects. An understanding of the regulation and chemical triggers of stem cell proliferation and differentiation are key to addressing birth defects. Additional research is needed to explore the therapeutic merits of cell transplantation techniques while accepting the likelihood that possible adverse side effects may occur. Therefore, improvements in specific isolation, purification and immune characterization of hUC-MSCs may facilitate clinical application.

**KEYWORDS:** MSCs (mesenchymal stem cells), UC(umbilical cord), hUC-MSCs (human umbilical cord mesenchymal stem cells)

**INTRODUCTION**

Stem cells are undifferentiated biological cells that can differentiate into specialized cells and can divide (through mitosis) to produce more stem cells. In mammals, there are two broad types of stem cells: embryonic stem cells, which are isolated from the inner cell mass of blastocysts, and adult stem cells, which are found in various tissues. Stem cells can also be taken from UCB just after birth.<sup>[1]</sup> MSCs are a heterogeneous subset of stromal stem cells, which can be isolated from the bone marrow, mobilized peripheral blood, cord blood, umbilical cord (UC), placenta, adipose tissue, dental pulp, and even the fetal liver and lungs. UC contains two umbilical arteries (UCAs) and one umbilical vein (UCV), both embedded within a specific mucous connective tissue, known as Wharton's jelly (WJ), which is covered by amniotic epithelium. UC is considered medical waste and the collection of UC-MSCs is noninvasive; furthermore, the access to UC-MSCs has not been encumbered with ethical problems.

There are several methods for collecting cord blood. The method most commonly used in clinical practice is the "closed technique", which is similar to standard blood collection techniques. With this method, the technician cannulates the vein of the severed umbilical cord using a needle that is connected to a blood bag, and cord blood flows through the needle into the bag. On average, the closed technique enables collection of about 75 ml of cord blood.

Collected cord blood is cryopreserved and then stored in a cord blood bank for future transplantation. A cord blood bank may be private (i.e. the blood is stored for and the costs paid by donor families) or public (i.e. stored and made available for use by unrelated donors).

Cord blood collection happens after the umbilical cord has been cut and is extracted from the fetal end of the cord, diverting up to 75 +/- 23 mL from the neonate. It is usually done within ten minutes of giving birth. Additional stem cells may be collected from the placenta. After the health care provider draws the cord blood from the placental end of the umbilical cord, the placenta is couriered to the stem cell laboratory, where it is processed for additional stem cells. An adequate cord blood collection requires at least 75mL in order to ensure that there will be enough cells to be used for a transplantation. Before the cord blood is stored for later use, it undergoes viral testing, including tests for HIV and Hepatitis B and C, and tissue typing to determine Human Leukocyte Antigen type. It will also be examined for nucleated cell count, cell viability, blood group antigen ABO & Rh blood group system, molecule cluster (CD<sup>34</sup>), and bacterial and fungal growth.<sup>[2]</sup>

**COLLECTION**

The umbilical cord was doubly clamped and transected 10 to 30sec after the delivery of newborns of 32 or more weeks of gestation. After removal of the newborn from the operative field, the free end of the cord was wiped

with betadine to ensure sterility of the collections. While the placenta was still *in utero*, the umbilical vein was punctured and the cord blood was collected by gravity in the collection bag. During cesarean sections or multiple births, after the birth of the baby, UCB was then collected from the removed placenta outside the operating theater. Efforts were made to obtain maximal volumes from each collection. The delivery of the newborn was not influenced in ed nor the delivery of the placenta delayed. After collection, 20 ml of venous blood were obtained from the mother and 3-5 cm of umbilical cord were taken to be frozen at -80°C. The UCB units were stored at 4°C, transported to the bank twice daily with the corresponding paperwork and processed within the following 24hr.

The volume of the cord blood collected was calculated from the weight of each collection minus the weight of the bag. Samples of 3 ml per unit were taken at this stage for HLA typing, a nucleated cell (NC) count, CD<sup>34+</sup> cell count and progenitor cell assays. Fungal, aerobic and anaerobic bacteriology cultures were performed on all donations.<sup>[3]</sup>

### ISOLATION

All samples were obtained after patients' consent. UCs were collected and processed within 24 h of natural delivery. Whole cord was washed in sterile phosphate buffered saline (PBS), three times to remove red blood cells, immersed in 70% ethanol for 30 s, and then immediately washed in PBS before further processing. Approximately 2-3 cm of whole cord was taken for processing as mixed cord, and approximately 6 cm of whole cord was dissected to obtain artery, vein, Wharton's jelly and cord lining. Explant cultures were obtained from each region which was weighed, minced into small pieces (~2 mm<sup>3</sup>) with a sterile scalpel, placed into 6 well plates, and grown in media containing: Dulbecco's Modified Eagle's Medium (DMEM F12), foetal calf serum (FCS), and penicillin and streptomycin (P/S). Tissue explants were removed after 21 days in culture. Adherent cells were passaged upon reaching 70% confluence and reseeded at  $5 \times 10^3/\text{cm}^2$  in either 25 cm<sup>2</sup> or 75 cm<sup>2</sup> tissue culture flasks for growth kinetics or for further culture expansion. Viable cells were counted by trypan blue (Sigma) exclusion in a haemocytometer. In addition, for mixed cord cultures the whole cord was cut into small pieces (~2 mm<sup>3</sup>) and digested with collagenase (1 mg/mL of type I) for 1 h at 37°C. Tissue was removed from the digest and the supernatant centrifuged at 80 g for 10 min; the pellet was then resuspended in 5 mL of the previous medium and plated in a 25 cm<sup>2</sup> tissue culture flask. Medium was changed every 2-3 days and cells were maintained in a humidified atmosphere at 5% CO<sub>2</sub> at 37°C.<sup>[4]</sup>

### Cryopreservation of Cord Blood

UCB processed units were cryopreserved using an automated microprocessor-controlled rate freezer. Briefly, after chilling of the WBC, ice-cold freezing

cryopreservative solution containing 60% DMSO was added dropwise for 15 min. Samples for quality control of cryopreservation procedure were extracted before freezing and cryopreserved into cryotubes with the bag. The cells were immediately placed in aluminium cassettes in the chamber of the cell freezer, which used two thermocouple probes placed in a sample ampule containing freezing media. The cryopreservation protocol was as follows: 1°C/min cooling down to -60°C, followed by a drop to -120°C, 5°C/min. At the end of the freezing procedure the cells were stored in the liquid phase of a liquid nitrogen freezer.<sup>[5]</sup>

### POTENTIAL USES

#### Cancers

Leukemia, lymphoma and several other cancer types are treated with cord blood stem cells. Cancer is a deadly condition for many patients, and often causes weight loss, fatigue and constant pain.

#### Blood Disorders

Blood disorders, including multiple types of anemia, are treated with cord blood stem cells. These conditions are caused by an imbalance in the body's cellular structure, and lead to swelling, blood clots and excessive bleeding, which can be deadly.

#### Metabolic disorders

Metabolic disorders, including Hurler syndrome and Krabbe disease, often lead to seizures, weight loss and frequent body pain. In some cases these diseases can be extremely dangerous.

#### Immune Disorders

Immune disorders, like Evans syndrome and Myelokathexis, will cause the body to attack its own cells. This often results in fatigue and constant fevers. In certain cases, an immune disorder can be deadly.

Clinical trials are currently testing cord blood as a treatment for other serious conditions, including: Autism, Hearing Loss, Cerebral Palsy, Spinal Cord Injury.

#### Cerebral ischemia

Several studies have reported in general that hUC-MSC transplantation into the cortex of an occluded middle cerebral artery can result in the successful in recovery of neurological function in a mice model. In this process have been shown that hUC-MSCs migrate to the ischemic area and differentiate into neurons, glial and other different types of neural cells. There appears to be increased cortical blood flow in the ischemic zone because of angiogenesis by hUC-MSCs.

#### Spinal cord injury

hUC-MSC transplantation can be a potential strategy for the promotion of corticospinal fiber regeneration and improvement in locomotor function following spinal cord transection into the lesion area of SCI rats compared with a control group. The results showed increased

numbers of regenerated axons in the corticospinal area of the zone around the SCI lesion. Migration of hUC-MSCs from the implantation zone was observed. This group of researchers hypothesized that the release of cytokines and growth factors from stem cells was the key mechanism for corticospinal fiber progression.

#### Alzheimer's disease

hUC-MSCs in cell culture medium and an AD mouse model can reduce and ameliorate disease symptoms. This group has reported that co-culture of hUC-MSCs with hippocampal neurons resulted in a significant reduction in apoptosis compared with the control group. Also they have shown that injection of A $\beta$  in the brain of a mice model that transplanted hUC-MSCs led to diminished oxidative stress and glial activation compared with the control group.

#### Support of hematopoiesis

MSCs have the potential capacity to support hematopoietic stem cell growth both *in vivo* and *in vitro*. hUC-MSCs could support the growth of CD34<sup>+</sup> cord blood cells as determined by long-term culture initiation cell culture (LTC-IC). In another study, researchers have shown that UC-MSC compared with BM had the capability to produce hematopoietic growth factors such as IL-6, IL-8, IL-11, G-CSF, GM-CSF and LIF. Researchers show that hUC-MSC increases homing and migration of UCB CD34<sup>+</sup> cells to the BM and spleen. According to evidence, there is low efficacy of UC-MSC hematopoietic support capacity compared with BM however UC-MSCs have the ability to support long-term hematopoiesis *in vitro*.

#### Autoimmune diseases

Immuno-modulatory effects of MSC have a critical role in the mechanism of autoimmune diseases. MSCs express low levels of HLA-I, but do not express HLA-II co-stimulatory molecules such as CD80, CD86 and CD40. In addition, these cells suppress activated T-cell proliferation and differentiation.

#### Rheumatoid arthritis

UC-MSCT is a new therapeutic method for treatment of RA. UC-MSCs with synovial tissue harvested from patients with RA, have shown that UC-MSC inhibited fibroblast-like synoviocytes (FLSs). FLSs, are resident cells of synovial joints that have a crucial role in inflammation and joint destruction. UC-MSCs suppressed the invasive behavior, MMP-9 expression and inflammatory response of FLSs. In addition, T cells that play an important role in maintenance of self immune tolerance in RA significantly increased. Assessment of UC-MSC on collagen-induced arthritis (CIA) in a mouse model explored that these cells prevented tissue damage, reduced inflammatory responses and Re-established the ratio between Th1 and Th2 cells. The results showed that, UC-MSCs significantly improved CIA in mice.

#### Wound healing

In a study of a mice model with a full skin defect, injection of hUC-MSCs in the wound site has shown significantly enhanced wound healing with a much thicker newly formed epidermis layer in the experimental group compared with the control group. A significant increase in the amount of cells was observed in the regenerated skin tissue with improved dermal ridges. In the experimental group, folliculus pili and other appendages had an important role in repairing skin tissue which was not observed in the control group. UC-MSCT caused more rapid, enhanced and improved wound healing.<sup>[6]</sup>

#### CONCLUSION

Stem cell therapy represents a fascinating new approach for the management of various diseases. Additional research is needed to explore the therapeutic merits of cell transplantation techniques while accepting the likelihood that possible adverse side effects may occur. Therefore, improvements in specific isolation, purification and immune characterization of hUC-MSCs may facilitate their clinical application. The promising results in clinical trial in treatment of patients with liver cirrhosis, systemic lupus erythematosus, multiple sclerosis and GVHD after hematopoietic stem cell transplantation for leukemia will further promote the therapeutic application in cell therapy and regenerative medicine.

Provided with the above-mentioned advantages, hUC-MSCs may become attractive cell sources in tissue engineering and treatment of currently refractory and degenerative diseases.

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