

Umbilical Cord Blood—Biology, Banking, and Therapeutic Use

a report by

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Umbilical cord blood (UCB) provides an alternative source of hematopoietic stem cells to bone marrow and peripheral blood for transplantation to reconstitute the blood and immune system following myeloablative or radiation therapy for numerous malignant and non-malignant conditions.¹ This year marks the 20th anniversary of the first UCB hematopoietic stem cell transplant. The first UCB transplant was conducted in 1988 to treat a patient with Fanconi anemia in Paris, France using UCB from a human leukocyte antigen (HLA)-matched sibling donor. The patient reached engraftment 22 days post-transplant with no complications of graft-versus-host disease (GvHD) and complete hematological and immunological reconstitution.² Since this first UCB transplant in 1988, there have been over 10,000 UCB transplants conducted worldwide.³ The use of UCB is expected to continue to grow as more UCB units are available for traditional hematopoietic transplantation and as new stem cell therapies such as regenerative medicine emerge into clinical application. This review summarizes UCB biology; the collection, processing, and storage methods for UCB banks; current clinical applications; and emerging clinical applications of UCB.

Cord Blood Biology

Many of the advantages of UCB for traditional transplantation and emerging applications are attributable to its collection at birth and resultant naïvety compared with bone marrow and peripheral blood, which are generally collected much later in life. UCB is primarily composed of monocytes and lymphocytes, which reside in the mononuclear cell (MNC) fraction. The lymphocyte population of UCB is immunologically immature compared with bone marrow and peripheral blood. UCB has a higher ratio of naïve versus memory CD4⁺ and CD8^{high} T cells, a higher concentration of natural killer (NK) cells, and a lower concentration of highly reactive NK T cells compared with bone marrow and peripheral blood.⁴ Cytokine expression in UCB is lower than in peripheral blood and bone marrow,² and UCB shows a greater expression of anti-inflammatory cytokines compared with pro-inflammatory cytokines. The immunologically immature characteristics and the anti-inflammatory properties of UCB are responsible for the low frequency and reduced severity of GvHD in allogeneic transplants, and may facilitate indirect cellular therapeutic benefits for regenerative medicine applications.^{4,5}

The largest stem cell population in UCB is of hematopoietic lineage expressing the cell surface glycoprotein CD34⁺. Approximately 1% of the MNC in UCB express CD34⁺.¹ The CD34⁺ cell population is heterogeneous, containing primitive cells and more mature cells further differentiated along the hematopoietic lineage. UCB has a higher percentage of primitive CD34⁺ cells such as pluripotent CD34⁺CD38⁻ and

CD34⁺CD33⁻ cells than bone marrow or peripheral blood.⁴ These primitive CD34⁺ cells are believed to be responsible for the long-term hematopoietic reconstitution in transplantation.^{4,6} UCB stem cells have a higher proliferative potential with reports of up to eight-fold greater proliferation rates than bone marrow stem cells,⁷ which may be due to longer chromosome telomeres in this young source of stem cells.⁸ In addition to hematopoietic stem cells, UCB has also been reported to contain endothelial cell precursors, mesenchymal cell progenitors, and multipotent–pluripotent stem cells, which may prove to be of high utility for regenerative medicine applications to treat conditions or damage affecting non-hematopoietic tissues.¹

Cord Blood Banking

The unique ability to easily collect and store UCB as a source of stem cells for transplantation, without harvest risk to the child or mother, led to the development of UCB banks in the 1990s.⁹ There are two types of UCB bank: public and private (or family) banks. Public UCB banks operate similarly to blood banks, where the UCB is donated to be stored for public use. There are approximately 35 public banks worldwide with a combined total of approximately 250,000 UCB units. In the US, there are 13 public banks containing approximately 87,000 units with locations in New York, Oregon, California, Colorado, North Carolina, Illinois, Florida, New Jersey, Washington, and Missouri.^{9,10} In the public UCB banking system, mothers wishing to donate must go through an intensive screening process for medical history, high-risk behaviors, infectious disease testing, and, in some cases, inherited disease testing. For the mothers who meet inclusion criteria, the collected UCB must also meet quality standards for cell count and volume prior to storage. It is estimated that 65–70% of collected units for public banks are discarded.¹⁰ There is no cost to donate the sample, but public banks charge transplant hospitals fees ranging from \$15,000 to \$35,000 to retrieve the sample.¹⁰ Private banks store the UCB for future use by the child from whom it was collected or a relative, such as a sibling. It is estimated that over 600,000 UCB units are stored in private banks worldwide.⁹ Reasons parents choose to bank their child's UCB include peace of mind, biological insurance, family history of a disease treatable by stem cells, adoption, mixed ethnicity, and as a genetically identical stem cell resource for future regenerative medicine applications for their child.

The UCB is collected by an obstetrician or midwife using collection kits provided by the bank. The kit is shipped to the bank for processing and storage. Private banks collect a fee of approximately \$1,500 for initial collection and then a storage fee of approximately \$100 per year. Private banks are open to all parents who wish to store since there are no

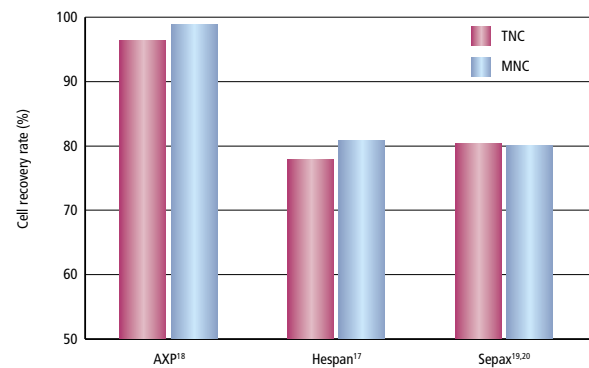
exclusion criteria and no geographical barriers. Maternal blood draws are tested for infectious diseases such as hepatitis B and C, human T-lymphotropic virus, cytomegalovirus, syphilis, and HIV; depending on the private bank, a positive infectious disease status may not prevent storage, but is kept on record. Generally, there is no cost to release the sample, but some private banks may charge for processing or shipment fees. Some private banks provide programs that allow cord blood storage for directed donation at no cost when a relative has a disease known to be treatable by stem cell transplantation. An example of a directed donation program is the Designated Transplant Program at the Cord Blood Registry® (Cbr Systems, Inc., San Bruno, CA), which has over 1,500 units stored for families.

Cord Blood Collection, Processing, and Preservation

UCB is generally collected following delivery either while the placenta is still *in utero* or after placental delivery. Collections while the placenta is *in utero* have higher volumes, a greater number of total nucleated cells (TNCs), more CD34+ cells, and more colony-forming units (CFUs).¹¹ The uterine contractions while the placenta is *in utero* are postulated to facilitate the blood draw, improving the collection volumes. With most methods, after the baby is delivered the umbilical cord is clamped, cut, and disinfected with an alcohol or betadine swab, and a needle is inserted into the umbilical vein. The blood can be collected by active syringe withdrawal or gravity bag. Collection volume, TNC, and MNC counts have been reported to be higher for the syringe-collected samples,¹² although experienced collectors typically collect comparable volumes with both collection methods and many obstetricians prefer the ease of the gravity bag. In addition to the collection method, the obstetric factors shown to be associated with greater stem cell yields are gestation time close to 40 weeks, heavier birth weight, moderate mother's age (20–37 years), and fewer previous pregnancies.¹³

After collection, UCB is transported to a processing center, where it is prepared for cryogenic storage. Most UCB banks reduce the unit volume by removal of excess red blood cells and plasma. This processing allows for reduced storage costs and space, increased post-thaw stem cell viability,¹⁴ and a reduced risk for blood type/antibodies blood group (ABO) complications for allogeneic transplantation.¹⁵ UCB processing must be performed with reproducibly high stem cell recovery and viability rates, ensuring that minimal stem cells are lost during processing. There are several processing methods, including hydroxyethyl starch sedimentation, density gradient centrifugation, and automated processes. The method currently used by most UCB banks is Hespan® (Abbott Laboratories, North Chicago, IL), which is a hydroxyethyl starch sedimentation process using a single centrifugation step to obtain a modified buffy coat product.¹⁶ The reported MNC recovery rate for Hespan is 80.9% and TNC recovery rate is 78.0%.¹⁷ Currently, the highest recovery rates published are for the AutoXpress Platform™ (AXP™) (ThermoGenesis Corp., Rancho Cordova, CA), an automatic standardized system used by the New York Blood Center Cord Blood Bank and Cord Blood Registry®, the largest public and family UCB banks in the world. The AXP platform recovers 98.7% of the MNC fraction and 96.2% of the TNC fraction,¹⁸ which is substantially higher than other automated systems such as Sepax™ (Biosafe, Eysins, Switzerland), which recovers 80.3% of the TNC fraction and 79.9% of the MNC fraction (see Figure 1).^{19,20}

Figure 1: Total Nucleated Cell and Mononuclear Cell Recovery Rates for Three Umbilical Cord Blood Processing Methods



Post-processing, UCB units are prepared for long-term storage under cryogenic conditions. The cells are frozen by a computer-controlled rate to cryogenic temperatures with the addition of a cryoprotectant agent (such as dimethylsulfoxide [DMSO]) to reduce cryoinjuries caused by cellular dehydration, ice crystallization, and osmotic changes. Generally, UCB is stored in liquid nitrogen or the vapor phase of the liquid nitrogen at -196°C. Specially designed freezers, called dewars, allow for storage in the vapor phase, preventing cross-contamination by viruses such as hepatitis and papilloma and other potential contaminants. The UCB storage container can be over-wrapped with soft plastic to provide an additional barrier to contamination. The longest study to date has shown that under proper storage conditions UCB stem cells did not have a significant loss of biologic activity (stem cell recovery, proliferation, viability, and transplant function) after 15 years of cryopreservation.²¹

Clinical Applications

UCB has been used successfully in hematopoietic stem cell transplantation to reconstitute the blood and immune system for malignant and non-malignant blood and bone marrow disorders. Conditions treatable with UCB stem cells include blood-related disorders, malignancies, and inherited metabolic and immune disorders (see Table 7). Brunstein and colleagues reviewed unrelated UCB transplant outcomes and found that disease-free survival rates (one to three years post-transplant) for leukemia patients ranged from 31 to 60% for children and from 19 to 77% for adults.²² For non-malignant conditions, survival rates for children undergoing unrelated UCB ranged from 70 to 80% for immunodeficiency diseases,²² and have been reported at 72% for metabolic storage diseases.²³ There have been fewer related UCB transplants conducted, and most have been for pediatric patients due to access to related UCB samples. One early study reported significantly higher survival rates for recipients of related UCB transplants compared with unrelated UCB transplants (63 versus 29%; $p < 0.001$) for the treatment of malignancies and non-malignancies.²⁴ Eurocord reported three-year related UCB transplant survival rates of 71% for early-phase, 45% for intermediate-phase, and 24% for advanced-phase malignancies, 82% for bone marrow failure disorders, 100% for hemoglobinopathies, and 70% for inborn errors of metabolism or primary immunodeficiencies.³ Factors associated with the outcome of UCB transplants include patient disease status, negative cytomegalovirus status, and cell dose in the graft.²⁵ The majority of studies have found that cell dose, measured as TNC, CD34+, or CFU granulocyte

Table 1: Diseases Treatable by Cord Blood Stem Cells

Blood-related disorders
Bone marrow failure disorders ⁵⁹
Hemoglobinopathies ⁶⁰
Histiocytic disorders ¹⁰
Myelodysplastic/myeloproliferative disorders ⁶¹
Platelet abnormalities ⁶²
Malignancies
Leukemias ⁵⁹
Lymphomas ^{59,63}
Plasma cell cancers ⁶⁴
Bone and soft-tissue sarcomas ⁵⁹
Solid tumors ³¹
Inherited metabolic and immune disorders
Leukodystrophies ^{23,65}
Lysosomal storage diseases ²³
Severe combined immunodeficiencies ¹⁰
Other primary immunodeficiencies. ¹⁰
Emerging stem cell applications
Type 1 diabetes ⁵⁴
Brain injury ⁵⁶
Autoimmune disorders ⁴³
Cardiovascular disease ⁴³

Table 2: Advantages and Disadvantages of Cord Blood

Advantages	Disadvantages
Simple harvest with no risk to donor	Cell dose
Long-term storage ability	One-time supply
Immediately available	Potential delayed engraftment time
Immunologically mature	
High concentration of stem cells	
High proliferative rate	
Younger cells with longer telomeres	
Low incidence of viral contamination	

macrophages (CFU-GMs), has the greatest impact on engraftment and subsequent transplant complications and survival.

Transplantation experience over the past 20 years has shown that UCB is associated with significantly fewer GvHD complications than bone marrow and peripheral blood transplants. This likely contributes to the growing use of UCB since chronic GvHD is the leading cause of non-relapse-related mortality in transplantation.²⁶ A meta-analysis of UCB and bone marrow transplantation for malignant and non-malignant diseases found similar survival rates but lower GvHD rates for children and adults, despite more HLA disparity between the UCB transplant donors and recipients.²⁷ In HLA-identical sibling donor transplantation, UCB has comparable survival rates but significantly less acute and chronic GvHD than bone marrow.²⁸ Related UCB transplantation appears to reduce the risk for GvHD (with reports ranging from 3 to 20% for acute and from 6 to 14% for chronic) compared with unrelated UCB transplants (with reports ranging from 20 to 50% for acute and from 5 to 30% for chronic).²² Interestingly, UCB transplant recipients have shown higher responses to treatment for chronic GvHD compared with bone marrow and peripheral blood transplant recipients.²⁹ Peripheral blood allogeneic transplantations show even higher rates of GvHD compared with bone marrow.³⁰

Autologous hematopoietic stem cell transplants have far fewer complications due to no risk of immune complications, and are often indicated in transplant situations when the disease has no genetic etiology. Indications for autologous transplantation include acute myeloid leukemia, many forms of non-Hodgkin's lymphoma, myeloma, solid tumors, and autoimmune diseases such as multiple sclerosis and Crohn's disease. Autologous cord blood transplants have been conducted successfully for the treatment of neuroblastoma,³¹ aplastic anemia,³² and lymphoblastic leukemia.³³ As the private UCB banking industry grows, the use of UCB in autologous transplantation is expected to grow as private banks provide readily available hematopoietic stem cells without complicated harvest for autologous transplant indications.

UCB offers several advantages for use in allogeneic and autologous transplantation over bone marrow and peripheral blood (see *Table 2*). As discussed above, UCB transplants have less GvHD, which allows for a higher immune tolerance of HLA disparities in allogeneic transplantation. Siblings are twice as likely to be able to use one another's UCB compared with bone marrow since UCB transplantation has been performed with one, two, or even three HLA mismatches,^{34,35} whereas bone marrow transplantation typically requires a perfect HLA match. UCB also has a larger number of CFU-GMs,³⁶ stem cells with longer telomeres and increased proliferative capacity. These characteristics appear to provide a more complete hematological and immunological reconstitution than bone marrow. A study by Frassoni and colleagues found that the bone marrow of children one year post-UCB transplantation had higher numbers of committed and early progenitor cells compared with the bone marrow of children one year post-bone marrow transplantation.³⁷ Additionally, significantly longer telomeres have been observed in the peripheral blood MNC cells in transplant recipients of UCB grafts compared with those receiving peripheral blood grafts, which suggests a replicative advantage for UCB stem cells.³⁸ UCB is associated with a lower risk for viral contamination compared with bone marrow.³⁹ The ease and safety of UCB collection and ability for long-term storage gives UCB the advantage of immediate availability for autologous and allogeneic clinical use, whereas the time needed to identify a bone marrow or peripheral blood donor, locate the donor, establish eligibility, and harvest the cells may result in disease relapse or progression precluding transplant feasibility.³

One disadvantage of UCB transplantation is low graft cell dose due to the limited collection volume available. Cell dose affects the time needed for hematological recovery. Several reports have shown that lower cell doses are associated with slower engraftment times.^{25,34,40,41} Cell dose is a larger obstacle in adult transplantation compared with pediatric transplantation due to the larger cell doses needed for hematological reconstitution of larger body masses. Expansion of UCB stem cells and the use of multiple UCB units are two proposed methods of circumventing the cell dose issue for adults. Despite the slow engraftment time, one study found that adult recipients of unrelated UCB transplants had similar engraftment, treatment-related mortality, and disease-free survival rates to those who received related bone marrow or peripheral blood.⁴²

Emerging Applications

Regenerative Medicine

UCB stem cells hold promise for regenerative medicine applications to treat damaged and diseased cells and tissues outside of the

hematopoietic lineage. Some of the conditions that are being researched in regenerative medicine applications include cardiovascular, endocrine, neurological, and orthopaedic disorders, many of which currently do not have effective medical treatments. Hematopoietic sources of stem cells overcome many of the barriers of embryonic stem cells since they are not associated with ethical controversies and are not tumorigenic.⁴³ Stem cells in hematopoietic sources are more accessible and found in higher concentrations than stem cells in other adult tissues such as adipose tissue and the brain. UCB has the added advantage over bone marrow and peripheral blood of a safe uncomplicated harvest and ability to store an autologous source of stem cells indefinitely from collection at birth.

UCB has several characteristics that make it attractive for use in regenerative medicine. It contains populations of stem cells other than hematopoietic stem cells such as endothelial, epithelial, mesenchymal, and pluripotent embryonic-like stem cells.⁴⁴ These populations have the ability to differentiate into a multitude of cell and tissue types *in vitro*.^{7,44,45} UCB stem cells are able to ‘home in’ on damaged tissues and to stimulate therapeutic improvements for many animal models of disease such as brain injury, diabetes, and myocardial infarction.⁴⁶⁻⁵¹ UCB stem cells may alleviate the damage by differentiating into the specific types of cells that are damaged, or by indirect therapeutic actions such as secreting anti-inflammatory, angiogenic, and chemotactic factors to repair the damaged tissue or protect it from future damage.^{52,53} UCB’s large population of naïve regulatory T lymphocytes and predominately anti-inflammatory cytokine excretion may hold therapeutic advantages over bone marrow and peripheral blood for treatment of autoimmune diseases.⁵⁴ These properties may help to combat autoimmune diseases, such as type 1 diabetes, by decreasing the inflammatory cytokine response and energizing the effector T cells that mediate the autoimmune attack.⁵⁴

UCB research in regenerative medicine is beginning to move from the bench toward the bedside. Two areas with incredible promise for UCB regenerative treatments are perinatal brain injury and type 1 diabetes. To date nearly 30 children have received autologous UCB units for investigational treatment of cerebral palsy and other brain injuries at Duke University (Cord Blood Registry®, personal communication). In January 2008, Duke University initiated a formal phase I clinical trial investigating the feasibility and safety of autologous UCB treatment for perinatal hypoxic brain injury.⁵⁵ The University of Florida, in collaboration with the Juvenile Diabetes Research Foundation and the National Institutes of Health, is conducting an interventional study testing the ability of autologous UCB infusions to treat type 1 diabetes in children.⁵⁶ Preliminary results for the first eight subjects to reach six months post-infusion show significantly lower daily insulin requirements and glycated hemoglobin (HbA_{1c}) values in the subjects receiving the autologous UCB infusion compared with age- and disease-duration-matched controls.⁵⁴ Family UCB banks provide their clients with the opportunity to participate in these exciting and potentially ground-breaking experimental treatments; without access to their stored autologous UCB, these children would not have access to these treatments. In turn, the family banks provide subjects to researchers who are developing and conducting regenerative medicine trials utilizing autologous cord blood that may change the course of medical treatment for numerous diseases.

Table 3: Obstetrician Patient Counseling—Key Points to Address for Umbilical Cord Blood Banking

UCB contains stem cells, which are the building blocks of the body’s blood and immune system and many other tissues.

UCB can be easily harvested after delivery without risk to the mother or baby.

UCB stem cells, similar to bone marrow stem cells, can be used to treat more than 70 conditions, including various cancers (e.g. leukemia and lymphoma), blood disorders (e.g. aplastic anemia), and genetic diseases (e.g. sickle cell disease).

UCB stem cells may be useful for the baby (the donor), siblings, parents, or cousins if there is an adequate immune compatibility (human leukocyte antigen match).

UCB stem cells have unique biological qualities compared with other stem cells that allow for a greater chance of use between family members and improved clinical outcomes in transplantation.

Depending on the delivering hospital, UCB can be donated to a public bank and made available to patients in need of transplantation without a matching related donor available.

Family banks provide collection and storage services (for a fee of approximately \$1,500 for collection and \$100 per year for storage) for parents wishing to store their child’s cord blood for the future use of the child or a family member.

Family banked UCB provides a perfect genetic match for the child from whom it was collected and may provide a valuable resource for emerging regenerative medicine and gene therapy applications.

Resources for more information: Parents Guide to Cord Blood Banks (parentsguidetocordblood.org), National Cord Blood Program, New York Blood Center (www.nationalcordbloodprogram.org), and Cord Blood Registry (www.cordblood.com).

Gene Therapy

UCB is an attractive candidate as a vector for gene transfer due to its attainability, proliferation rate, and engraftment potential. Gene therapy using autologous stem cells has the added benefit of none of the immune complications associated with allogeneic transplant. To date, trials have shown gene therapy using hematopoietic stem cells to be beneficial for patients with childhood immunological diseases such as cross-linked and adenosine deaminase severe combined immunodeficiency diseases (SCID) and chronic granulomatous disease.⁵⁷ Other gene therapy targets include hemoglobinopathies such as sickle cell disease and thalassemia.⁵⁸ Current research is focused on preventing the serious side effects that were seen in the first clinical trials.^{57,58}

Conclusion

UCB is increasingly being utilized for traditional hematopoietic stem cell transplantations and it appears to be an ideal candidate for emerging stem cell therapies in regenerative medicine and gene therapy. UCB has several advantages over other hematopoietic stem cell sources such as being easily harvested without risk to donor, providing younger cells with longer telomeres, increased proliferative capacity, reduced GvHD, being a perfect stored genetic match for autologous use, and being readily available for clinical use. UCB can easily be collected at birth and stored for future use by the public at a public bank or for the family or child from whom it was collected at a private bank. The availability of autologous UCB units stored at private banks has allowed their clients to participate in ground-breaking experimental regenerative medical treatments, currently available only to children with UCB stored. Given the medical significance of UCB, it is essential that obstetricians inform and discuss with their expecting patients the options of private and public UCB banking. *Table 3* provides some key points for obstetricians to address with their patients and resources for more information on UCB banking. ■

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