Frontiers of Cord Blood Science
Niranjan Bhattacharya · Phillip Stubblefield
Editors

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Foreword by Eliane Gluckman
Evidence supporting the efficacy of umbilical cord blood hematopoietic stem cells for allogeneic transplantation has significantly increased over the past years, as it now becomes a standard alternative to bone marrow transplantation in many centers. Since the first cord blood transplant successfully performed in a child with Fanconi anemia with his HLA-identical sibling, the number of allogeneic unrelated and related transplants performed for various hematological disorders is increasing steadily. To perform these transplants, cord blood banks are dedicated for collecting, cryopreserving, and performing searches for international exchanges. More than 8000 unrelated cord blood transplants have been performed worldwide; the transplants have been provided by cord blood banks that have collected more than 200,000 cord blood units. Cord blood cells have proliferative advantage and decreased immune reactivity when they are compared to adult bone marrow cells. These properties give a clear advantage for engraftment and diminution of graft-versus-host disease. In consequence, several studies have shown that cell dose and HLA are important factors for survival after transplant. The best units should contain more than $2 \times 10^7$ nucleated cells per kilogram and more than $2 \times 10^5$ CD34$^+$ cells per kilogram, the number of HLA mismatches should not be superior to 2. The advantages of cord blood compared to bone marrow transplants are the absence of risk to the donor, the direct availability of the cells, and the absence of infectious disease at birth. Further research are currently undertaken to improve the results of allogeneic hematopoietic stem cell transplants: they include the use of double cord blood transplants, the preparative regimen with non-myeloablative drugs, and the in vitro expansion of progenitor cells.

In addition to the presence of hematopoietic progenitors, it is now known when cord blood contains stem cells that have retained embryonic properties, they can be isolated, and, when cultured in appropriate conditions, give rise to cell lines that can be used for tissue engineering and regeneration of non-hematopoietic organs or tissues. Already, various cell lines have been generated from cord blood, including mesenchymal cells, endothelial cells, hepatocytes, muscle, cardiac myoblasts, pancreatic islets, keratinocytes, and neuronal cells. These results are very promising, but more research is needed before large-scale production for clinical use. Comparison with adult and embryonic stem cells will determine the best source for each
indication. Considering the availability of cord blood and the absence of ethical problems, cord blood will probably become the best source for cell therapy.

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Francis Bacon once wrote that science progresses by a succession of small steps through a fog through which even the keen sighted explorer can seldom see more than few paces ahead; occasionally, the fog lifts and an eminence is gained, and scientific truth gets kaleidoscopically rearranged into fact and fiction. In the year 1988, a 6-year-old boy from North Carolina with Fanconi anemia was transplanted with HLA-matched umbilical cord blood from his baby sister by Prof. Elaine Gluckman in Paris. Nobody at that time dreamt of the enormous possibilities of such an experimentation (Gluckman E, et al. Hematopoietic reconstitution in a patient with Fanconi’s anemia by means of umbilical cord blood from a HLA-identical sibling. N. Engl. J. Med. 1989;321:1174–1178). Most scientists and physicians were highly skeptical, doubting that a few ounces of cord blood contained sufficient stem and progenitor cells to rescue bone marrow after myeloablative therapy. However, this child engrafted without incident, and his blood, bone marrow, and immune system were fully regenerated with donor cells. He remains well and durably engrafted with donor cells 17 years following the original transplant.

Since the first successful transplantation of umbilical cord blood in 1988, cord blood has become an important source of hematopoietic stem and progenitor cells for the treatment of blood and genetic disorders. Significant progress has been accompanied by challenges for scientists, ethicists, and health policy makers. With the recent recognition of the need for a national system for the collection, banking, distribution, and use of cord blood and the increasing focus on cord blood as an alternative to embryos as a source of tissue for regenerative medicine, cord blood has garnered significant attention. Cells from cord blood have been shown to transdifferentiate into non-hematopoietic cells, including those of the brain, heart, liver, pancreas, bone, and cartilage, in tissue culture, and in animal systems (Porada GA, Porada C, Zanjani ED. The fetal sheep: a unique model system for assessing the full differentiative potential of human stem cells. Yonsei Med. J. 2004;45(Suppl):7–14). Recently, it has been demonstrated that both cardiac and glial cell differentiation of cord blood donor cells occurred in recipients of unrelated donor cord blood transplantation as part of a treatment regime for Krabbe disease and Sanfilippo syndrome (Hall J, Crapnell KB, Staba S, Kurtzberg J. Isolation of oligodendrocyte precursors from umbilical cord blood [abstract] Biol. Blood Marrow Transplant. 2004;10:67.; Crapnell KB, Turner K, Hall JG, Staba SL, Kurtzberg J. Umbilical cord blood cells
engraft and differentiate in cardiac tissues after human transplantation [abstract] Blood. 2003;102:153b). These observations raise the possibility that cord blood may serve as a source of cells to facilitate tissue repair and regeneration in the future. While this is purely speculative at this time, developments over the next decade are expected to clarify the potential role of both allogeneic and autologous cord blood in this emerging field.

Most of the work in this field has been with cord blood stem cells, which constitute only 0.01% of the nucleated cells in umbilical cord blood. The utility of the other cells which constitute 99.9% of umbilical cord whole blood has not been properly studied. Cord blood is a rich source of fetal hemoglobin, growth factor, cytokine-rich plasma as well as other nucleated cells, of which stem cells are an important constituent.

Transfusion of blood and other blood products has made possible many of the advances of modern surgery. Without the ability to safely give blood during many of the complex surgical procedures that have saved countless lives, these procedures would not have succeeded. For the last 70 years since the publication of the report of Amberson, there have been global attempts to find a genuine blood substitute. In a report of the World Health Organization, it was revealed that there are about 500,000 pregnancy-related deaths globally, of which at least 25% maternal deaths are due to the loss of blood. An estimated 13 million units of blood worldwide are not tested against human immunodeficiency viruses or hepatitis viruses, and in some developing countries 80% of the blood supply comes from paid donors or replacement donors (family, friends, or acquaintances) even when the infected population is high.

The current generation of blood substitutes can transport oxygen to tissues, and there are agents to replace platelets, plasma coagulation factors, and its various combinations. However, none of the attempts to provide a hemoglobin-based oxygen carrier, be it from a human or a bovine source of hemoglobin, has passed through the Phase III clinical trials in the United States. Apart from this, there are other issues of forbidding costs and complications.

It is a known fact that aseptically collected and properly screened human cord blood is pure, that is, free from bacteria, virus, protozoal contamination, in case of healthy newborn babies, as the cord blood passes through the finest biological sieve, i.e., the placenta. This blood has a much higher hemoglobin, platelet, and leukocyte content than adult whole blood. Additionally, it has a high concentration of cytokine/growth factors in its plasma, which eventually helps in the gene-switching mechanism after the birth of the baby. This blood also has a much higher oxygen-carrying capacity, and hence, the transfusion of fetal hemoglobin-rich cord blood may lead to better tissue perfusion of oxygen (vol/vol) to the recipient’s tissue than an identical volume of adult whole blood. Patients with severe anemia, renal failure, and other conditions of low cardio-respiratory reserve or tissue hypoxic condition in any age group might benefit from cord blood transfusion. This is especially important in high-risk cases with varying degrees of bone marrow senescence or failure due to any etiology. Here, CD34 stem cell-rich umbilical cord whole blood transfusion has the potential to have an immediate benefit of better tissue oxygenation
with an additional delayed benefit of possible engraftment of umbilical cord stem cells. These stem cells may prove capable of the rejuvenation of the bone marrow in case of structural or functional immunodeficiency as is the experience of transfusion in certain advanced cancers. Finally, another interesting aspect is the possibility of augmentation of surgical wound healing by the growth factor/cytokine-rich umbilical cord blood plasma, and use of cells from cord blood to coat synthetic grafts for reconstructive surgery, allowing their better adhesion.

There is a famous saying which is also very practical, “All that glitters is not gold”. Only time will prove whether these are mere hype or a true reality. With over 100 million births globally each year, more than 40 million units (250 ml) of human umbilical cord blood are produced, the vast majority of which is totally discarded as trash. With the ethical constraints surrounding the use of embryonic and fetal-derived stem cell sources, human umbilical cord blood represents the world’s greatest untapped resource for interventional therapy.
Acknowledgments

The editors give profuse thanks to Prof. S. Arulkumaran of London University, and currently the Secretary General of FIGO, and Dr. Himangsu Basu, former executive board member of the Royal College of the Obstetricians and Gynaecologists, UK, for their keen interest, advice, and support.

The editors are particularly grateful to Mr. John Harrison for his suggestion and guidance. Suggestions for improvement and advice from colleagues around the world, just to name a few, like Prof. Elaine Gluckman of Paris who is extremely kind to write the foreword of this book, Prof. Terry Storm of Harvard University, Prof. Linda Heffner, Prof. Anderson Deborah of Boston University, Prof. Hal Broxmeyer of the University of Indiana School of Medicine, and Prof. Ian McNiece of Johns Hopkins University. The editors also gratefully acknowledge the contributions of all the authors who took precious time from their busy schedules in order to help us to complete the book in time. The editors are also grateful to their wives (Prof. Sanjukta Bhattacharya for Dr. Niranjan Bhattacharya, and Linda Stubblefield, MSW, for Prof. Phillip Stubblefield) for their encouragement, understanding, and forbearance. With their own commitment in their respective fields, it is no surprise that their affection for the book would be less than that of ours but they tolerated and indulged in spite of the time subtracted from family activities. There are also innumerable patients, students, friends who have facilitated our work immeasurably. May God bless all of them for their support of cord blood science.

Dr. Niranjan Bhattacharya
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Introduction

In the animal kingdom, swallowing the afterbirth by the mother is a general norm. Nature appears to have provided this precious wisdom to some of its creatures. Even herbivorous animals swallow the placenta after the birth of their babies (for example, the cow). But humans, for the most part, do not appear to know about the potential use of this precious afterbirth, which has protected and nurtured the baby for so long in the womb. In traditional Chinese medicine, however, the dried human placenta (zi he che) has been used for therapeutic purposes for over thousands of years. According to this system of medicine, the placental biological property has been described as being sweet and salty in flavor, warm in nature, and it has been noted that it is tropistic to the heart, lung, and kidney channels. Being sweet, salty, warm, and moist in nature and mild in action, it functions in strengthening ‘Yang’, reinforcing essence and Qi, and nourishing blood, and is used as an excellent tonic. The dried human placenta, combined with other herbs, serves to treat syndromes of insufficiency of Yin, Yang, Qi, and blood.

In the rest of the world, however, there is little knowledge about the therapeutic properties of the human placenta. Of late, particularly since 1989, global consciousness is increasing, not on the use of the placenta per se but on the use of umbilical cord blood stem cells as an easily available source of hematopoietic stem cells for bone marrow transplantation. The first cord blood transplantation was done in Paris by Prof. Elaine Gluckman, a dedicated scientist, who has very kindly written the foreword to the present volume. Her pioneering work marked the initiation of the clinical application of cord blood. In the years following, cord blood transplantation has been successful, particularly in children with hematological, immunological, metabolic, and neoplastic diseases. Cord blood as an alternative source for hematopoietic progenitor cells (HPC) has several advantages, including rapid availability and lower risk of graft-versus-host disease (GVHD) despite human leukocyte antigen (HLA) disparity.

In cases where there is a non-availability of bone marrow or where the cord blood cell count is very low or where there is extensive myoablution due to drug, radiation or both, problems may occur in the host undergoing the transplant procedure. In such cases, multiple cord blood units or in vivo expansion of cord blood cells can provide a solution, with sophisticated infrastructural support. For example, many older patients, or those with extensive prior therapy or serious co-morbidities, are unable to tolerate conventional myeloablative conditioning.
Currently, research is on-going all over the world, which may improve the effectiveness of cord blood transplantation for the treatment of a variety of conditions, including non-myeloblative regimens; the use of ex vivo expansion to increase the numbers of HPCs; the development of new approaches to the acceleration of immune recovery; the use of multiple units of cord blood in transplantation; and facilitation of the upregulation of homing receptors. Attempts are being also made to clarify the existing scientific confusion about the transdifferential property of mesenchymal stem cells (MSC). The primary reason for the current interest in the use of cord blood is because of the stem cells which are found in cord blood and their trans-differentiation abilities, which have clinical implications in degenerative diseases. Hence, cord blood research is related to stem cell research. These fetal stem cells (CD 34), which are inherent in cord blood, cause less graft-versus-host reactions after transplantation. Recognition of this potentiality in the scientific world has resulted in the collection and harvesting of these cord blood stem cells in many laboratories all over the world. However, these hematopoietic stem cells constitute only 0.01% of the nucleated cells of the cord blood. The rest, i.e., 99.99% of the cord blood is not used for the most part. This wasted precious gift of Mother Nature is rich in fetal hemoglobin, growth factors, and other cytokine filled plasma, and is moreover protected in the infection-free environment inside the placenta in case of a healthy newborn. It, too, has great potentials, and it has been shown that it can be used not only as a safe alternative to adult blood for transfusion, but also has the added advantage of its various properties, which actually help in the all-round development of a neonate. This is significant because for years a search has been going on for a suitable hemoglobin-based oxygen carrier, and chemically or genetically modified bovine RBC or hemoglobin extracted from sea creatures like the sea worm (*Arenicola merina*) have been tried out for the purpose.

As a new human grows and develops inside its mother, blood is made to circulate to all the vital organs bringing nutrients from the mother to the fetus and carrying waste products back to the mother. Fetal blood in the placenta exchanges waste products from the baby for nutrients and oxygen from the mother and carries the ‘good stuff’ from the mother back to the fetus through one large vessel, the umbilical vein. Roughly one-third to 40% of all blood that the fetus makes is outside the fetus at any point of time, flowing to the mother or coming back from the mother. In pregnancy, there are dramatic and continuous molecular and biochemical changes with the progression of the gestation both for the fetus and the mother because there are two distinct separate genomes operating under the same organism (the mother). There are two distinct lines of blood supply with a unique interface (trophoblast) which have the specific functions of anchoring, controlling, transporting and metabolizing the specific need for the growth and maturation of the fetus, for that gestational state of growth and maturation. There are direct and indirect interactions between the mother, the embryo or the fetus, the placenta, the extra-amniotic membrane and the amniotic fluid. And the cord blood carries all the nutrition to meet the growth requirements of the organism at that specific gestational age.

The major emphasis of research internationally, however, is not on the transfusion of cord blood, but on the use of umbilical cord blood stem cells. These have a different set of microenvironmental exposures compared to those of adult
marrow or peripheral blood stem cells. The placenta is a complex organ that regulates feto-maternal interactions. Many cytokines that can influence lymphohematopoietic development, i.e., granulocyte colony stimulating factor (G-CSF), c-kit ligand (stem cell factor), granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin-15 (IL-15), all are produced by the placenta. The red cell collected from cord blood of the new born differs from the adult RBC in many ways. For example, there is an increase in the immunoreactive myosin of the red cell membrane [1], and the total value of the lipid, phospholipid, and cholesterol are more in proportion than the red cells of the adult [2].

Even the antigen expression of cord blood RBC differs from the adult RBC, viz., the A, B, S and Lutheran antigens are expressed in lesser amounts in cord blood than in adult RBC and there is a complete absence of Lewis antigens in cord blood [3]. Of greater interest, however, is the fact that there are fundamental metabolic differences between adult and cord blood RBCs, for example, the activities of phosphoglycerate kinase, enolase, glyceraldehydes-3- phosphate dehydrogenase, glucose phosphate isomerase, etc., which are the essential enzymes of the Embden-Meyerhof pathway, are definitely increased for the cell age in case of cord blood when compared with the adult RBC activities of those enzymes [4]. Apart from the enzymes mentioned above, the activity of a number of non-glycolytic enzymes is different in cord blood, viz., carbonic anhydrase, and acetylcholine esterase, only to name the essential few [5].

The full potential for the use of placental umbilical cord blood is as yet unknown. This volume is a compilation of the work of distinguished investigators who are currently engaged in discovering the latent possibilities of cord blood in the treatment of various diseases. The contributors summarize the current state of research in the field and discuss the potential future applications of cord blood both for research and for the treatment of different diseases and conditions. There are four chapters which are mainly hypothetical, one of which is a preview of the clinical uses of cord blood (Part III, Chapter 13). One is on the use of cord blood in strokes and other neurological disorders (Part III, Chapter 11), another is on the bioengineering applications of cord blood to improve the biofriendliness of a mechanical prosthesis or implant (Part V, Chapter 16), and the fourth one is also on how to increase the biofriendliness of a ceramic prosthesis with the use of cord blood MSCs and other cells (Part V, Chapter 17). There is one chapter on umbilical cord whole blood transfusion, based on actual clinical human trials in chronic anemias of several diseases to combat anemia, which is a co-morbidity in many chronic serious diseases (Part III, Chapter 10). There are 13 chapters dealing with the basic science and transplantation, preservation, in vivo expansion, and other aspects of cord blood stem cell application. And finally, one investigator has explored the ethics of cord blood research (Part VI).

**Perspectives on Cord Blood Banking and Cord Blood Stem Cell Transplantation**

There are several interesting observations on the transplantation of cord blood stem cells in this volume from different parts of the world like the USA, the UK,
Australia, Germany, Hong Kong, etc. Dr Anjali Mehta and colleagues noted that the placenta may prove to be a non-controversial source of hematopoietic and MSCs as well as endothelial progenitor cells (Part I, Chapter 1). A ‘cocktail’ of these three elements might be used in the future to treat hypoxic ischemic encephalopathy (HIE) in the peripartum period for neuroregeneration, and a combination of these cells might also be used to treat one of the more than 80 diseases that have responded to stem cell transplantation. Furthermore, these cells have the potential to treat degenerative diseases, such as heart disease, endocrine disorders like diabetes, and neurodegenerative diseases such as stroke, Alzheimer’s disease, Parkinson’s disease, and spinal cord injuries. These cells may also be useful in the treatment of orthopedic problems.

Further insight into the subject was has been provided by Dr. Peter Hollands of the UK Cord Blood Bank (Part I, Chapter 2) who has the experience of more than two decades in this field. He presents the advantages and disadvantages of cord blood as a source of stem cells.

The advantages in the use of cord blood as a source of stem cells for transplant are:

- Ease of procurement, processing, and storage
- No risk to donors
- Reduced risk of transmitting infection
- Immediate availability of cryopreserved units
- Acceptable partial HLA mismatches (4/6 HLA match)

Holland notes an added advantage that cord blood stem cells do not carry any of the legal, moral, ethical or religious objections associated with the use of embryonic stem cells.

The current disadvantages in the use of cord blood stem cells as a source of stem cells for transplant are:

- The limited number of hematopoietic stem cells in a cord blood unit which may lead to failed or delayed hematopoietic reconstitution or restricted use in adults
- Possible abnormalities in cord blood stem cells, e.g., early malignant mutations, which may have an effect on recipients
- It is not possible to collect additional donor stem cells, or donor lymphocytes, for those recipients who relapse following cord blood stem cell transplant.

For the purpose of easy availability of cord blood stem cells, cord blood banks have sprung up in different parts of the world. There are problems and controversies associated with cord blood banking. Dr. G.N. Stacey, the director of the UK Stem Cell Bank, has discussed the problems and advantages of different stem cell lines as practiced in UK Stem Cell Bank and mentions how others can use the facilities provided by the bank (Part V, Chapter 16). Prof. Stacey points out that the embryonic stem cell lines established in the UK are required, under the licence for derivation from the Human Fertilization and Embryology Authority, to be deposited in the UK Stem Cell Bank. Other groups working on the derivation of adult and non-UK hES cells are also very welcome to use the UK Stem Cell Bank facility.
Donation of cell lines into the bank is initiated by submission of information on the lines to the bank’s Steering Committee using the forms available on the bank and Medical Research Council websites. Confirmation by the Steering Committee that the cells meet ethical requirements for the UK and other scientific and technical criteria then activates the depositing process with the bank and establishment of the transfer agreements between depositor and the bank. There is also a generic agreement between the depositor and any institution receiving cells from the bank, to protect the depositor’s intellectual property in the cells.

In another chapter on cord blood banking and the controversies involved therein by Dr. Carolyn Troeger and colleagues, Switzerland (Part V, Chapter 17). According to them, cord blood stem cells are increasingly being used to repopulate bone marrow in the treatment of malignant and non-malignant diseases in children and adults. This development, however, is also related to a continuing debate on the role of public versus private cord blood banks. Public cord blood banks store HSC for allogeneic, usually unrelated, transplantations. Currently between 175,000 and 200,000 units are stored frozen worldwide. The infant and its parents donate the cord blood to the bank and therewith to the public. Unlike private cord blood banks, public banks do not charge for collection and storage. Most of the transplants are used for the allogeneic treatment of leukemia, about a quarter for the treatment of genetic diseases. Similarly, a related allogeneic HSC transplantation using the stored cord blood from a sibling is well established in public banks. Public cord blood banks have the opportunity to provide HSC also for ethnic minorities that are under-represented in bone marrow registries.

There are several chapters on the clinical applications of cord blood transplantation. Prof. Patricia Pranke and Dr. Raquel Canabarro from Brazil have suggested that umbilical cord blood stem cells are an efficient alternative for the transplantation of HPCs (Part I, Chapter 3). Parameters commonly used to evaluate an umbilical cord blood unit and predict transplant outcomes have been total nucleated cells and CD34+ cells counts. Lack of CD38, HLA-DR and lineage committed antigens, as well as the co-expression of Thy-1 (CDw90), c-kit receptor (CD117), among others surface markers, have been shown to identify the hematopoietic stem cells in umbilical cord blood. A number of factors can influence the volume and amount of CD34+ cells, which are considered as immature and capable of proliferation. Quantification of CD34+ as well as correlations of such factors as maternal age, gestational age, newborn sex and weight, umbilical cord length, placental weight with increased volume and concentration of immature cells, among others, are important issues that can influence the success of umbilical cord blood cells transplantation. According to the authors, ex vivo culture is a crucial component of several clinical applications currently in development including gene therapy, and stem/progenitor cell expansion.

Dr. Karen Quillen, Director of the Boston University Blood Bank, has done a retrospective comparison of 113 cord blood transplants and 2052 marrow transplants in children (15 years of age or younger), for the period 1990–1997 (Part II, Chapter 8). Over half the patients were transplanted for malignancy, most commonly acute leukemia. The recipients of cord blood were younger (median age of 5 years
versus 8 years) and smaller (median weight of 17 kg versus 26 kg) than the recipients of bone marrow. The median cell dose was $4.7 \times 10^7$ cells/kg for cord blood as compared to $3.5 \times 10^8$ cells/g for marrow. Despite less intense GVHD prophylaxis (with the omission of methotrexate in 72% of cord blood recipients), cord blood transplants had a lower risk of acute GVHD (14% versus 24%) and chronic GVHD (5% versus 14%). Hematopoietic recovery in terms of neutrophil (26 versus 18 days) and platelet (44 days versus 24 days) engraftment was significantly slower for cord blood transplantation compared to marrow. Interestingly, the three-year survival rate in patients transplanted for malignancy was similar in the two groups (46% for cord blood recipients, 55% for marrow recipients).

Prof. Karen K. Ballen of Harvard University comments in her review that over the last 17 years, there has been a dramatic growth in the use of cord blood as an alternative stem cell source for patients without matched related or unrelated bone marrow donors (Part II, Chapter 9). Initially, the majority of transplants were performed in children. Recently, the results in adult cord blood transplantation, according to her, appeared promising. She has discussed the outcome data for adult cord blood transplantation, with an emphasis on new techniques using double or sequential cord blood transplantation in her article. She has noted that cord blood CD34$^+$ cells in culture increase in cell number every 7–10 days, several hundred-fold greater than the increase in cultures of similar cells from adult bone marrow, thereby allowing a 10-fold lower CD34$^+$ cell dose to be used for successful cord blood transplantation. Moreover, cord blood cells have greater proliferative capacity and longer telomere length has been proposed as a possible explanation. Ballen further mentions that decreased GVHD following cord blood transplantation with preservation of a graft-versus-leukemia effect may be because the immunologic properties of cord blood differ from mature bone marrow or peripheral blood stem cells. Cord blood contains a high proportion of ‘naïve’ phenotype T cells that are CD45RA$^+$/CD45RO$^-$, CD62L$^+$. The chemokine receptor CCR5, expressed by TH1 T cells, is less abundant among cord blood T cells than adult T cells. She notes that cord blood T cell receptors when compared to adult blood T cell receptors, have a less complex repertoire. Cord blood cells express T cells with less clonal diversity than expressed by adult peripheral blood.

Prof. Shaw and his group from Australia state that the majority of hematopoietic stem cells transplants (HSCT) are done for patients with malignant diseases (Part II, Chapter 7). But, at the same time, it should be remembered that a high proportion of paediatric transplants are done for non-malignant disease, particularly true for unrelated transplants. HSCT is a curative option for many inherited and acquired non-malignant diseases that show abnormalities of the blood or bone marrow-derived cells. Current experience shows that unrelated CBT is a feasible procedure for children with bone marrow failure with the background of inborn errors, immunodeficiencies, hemoglobinopathy, etc.

Key researchers in the field such as Prof. Ian K. McNiece and Dr. Elizabeth J. Shpall of Johns Hopkins University, Baltimore, USA, have noted that cord blood products contain similar cell populations to bone marrow and mobilized peripheral blood progenitor cell products (PBPC), including hematopoietic stem cells (HSC), primitive progenitor cells, mature progenitor cells, and mature functional
cells (Part I, Chapter 4). However, the total cell number and progenitor cells are much lower in cord blood compared to bone marrow and PBPC. For example, bone marrow and PBPC contain approx $10^8$ CD34$^+$ cells while cord blood contains approximately $5 \times 10^6$ CD34$^+$ cells. In contrast, the frequency of HSC, as determined by NOD/SCID engraftment, is enriched in the CD34$^+$ cell population of cord blood compared to bone marrow or PBPC. As few as 100,000 cord blood CD34$^+$ cells can engraft NOD/SCID mice, while approx 1 million bone marrow CD34$^+$ cells and 5 million PBPC CD34$^+$ cells are required for engraftment of human cells. McNiece and colleagues noted that these numbers suggest that cord blood contains similar levels of HSC to bone marrow and PBPC, but significantly lower levels of committed progenitor cells.

Dr. Karen Bieback of the University of Heidelberg, Germany, has commented that: for allogeneic use, the primitive umbilical cord blood derived non-hematopoietic stem cell population provides perhaps the most readily accessible and at the same time, underutilized stem cell source, with little ethical conflict and a number of advantages (Part I, Chapter 6). However, the question as to whether cord blood progenitor cells will prove to be superior or more practical in terms of allogeneic use when compared to other adult tissue derived progenitor cells, remains open at this time. Nevertheless, efficient and reproducible methods to isolate, expand and differentiate cord blood progenitor cells are required. When extrapolated to the developments in hematopoietic stem cell transplantation, where within 10 years cord blood has become an increasingly accepted alternative source for HSC, Bieback is of the opinion that probably as many years will go by before the eventual use of cord blood-derived non-hematopoietic stem cells in clinical therapy.

In addition to hematopoietic stem cells, on which the investigators mentioned so far have written, it has been long recognized that the post-natal bone marrow in mammals is populated by a distinct stem cell population known as MSCs, also referred as marrow somatic cells or colony forming unit fibroblastic cells. In vivo, MSCs and other non-hematopoietic cells (e.g. macrophages, reticular cells, endothelial cells, smooth muscle cells, adipocytes) in combination with the extra cellular matrix, form what is known as a “hematopoietic inductive microenvironment”. This complex bone marrow microenvironment provides support for hematopoiesis, i.e., the process of formation of new blood cells, through cell-to-cell interactions and soluble clues. However, MSCs are not exclusively located in the bone marrow and are found in various other tissues, including the cord blood, fetal blood and liver, amniotic fluid and, in some circumstances, in adult peripheral blood.

Prof Steve Stice and Dr Pablo Bosch, from the University of Georgia, USA, report that MSCs can be isolated from several tissues (Part I, Chapter 6). MSCs are a fairly rare and unique cell type, and form, at most, 0.01% of adult human bone marrow. This is true for cord blood as well. However, more MSC cells exists within the umbilical vein endothelial/subendothelial layer. MSCs are considered pluripotent cells with a capacity to differentiate into mesodermal lineages, such as adipocytes, osteoblasts and chondrocytes both in vivo and in vitro. Several studies have demonstrated that these pluripotent stem cells derived from the marrow stroma, can be easily isolated from bone marrow specimens, proliferate extensively ex vivo to originate relatively homogeneous cell populations and are endowed with
the capacity to differentiate into mesodermal and non-mesodermal cell types. Due to their multipotentiality and extensive self-renewal capacity, MSCs hold great promise as a source of cells for many cell-based strategies for the treatment of human diseases. This chapter presents an in-depth description of the biology, isolation, characterization and potential therapeutic applications of MSCs.

Prof. Andrew Burd, Dr. T. Ayyappan, Dr. Lin Huang, representing the Chinese University of Hong Kong, China, are currently working on the problem of chronic wound healing (Part III, Chapter 12). They opine that the chronic wound becomes more vascular and can then be definitively closed with a skin graft. They cite a report on the use of allogenic bone marrow MSCs for the treatment of a patient with deep skin burns. Burd et al. feel that it is in such cases that there is a great potential for topical application of cord blood as a biological wound healing modulator. They further note that amniotic membranes have been used in the past as biological dressings for wounds but comment that concerns about risks of disease transmission have severely limited this practice in many parts of the world. Similarly, the question of potential risk of disease transmission when using cord blood may also be raised. However, they feel that there are already well defined screening processes to reduce and/or eliminate such risks as applied in routine blood banking. They assert that as the understanding of the range and nature of the stem cell composition in cord blood becomes more clear, it may be possible to apply more selective fractions onto wounds, both chronic and acute, to modulate the biological healing mechanisms, and an additional benefit is that it will be cost effective.

Perspectives on Cord Blood Transfusion

In a report of the World Health Organization, it was revealed that there are about 500,000 pregnancy related deaths globally, of which at least 25% maternal deaths are due to the loss of blood [6]. One of the most important advances in surgery has been the availability of blood and other blood products. Without the ability to safely give blood during many of the complex surgical procedures that have saved countless lives, these procedures would not have succeeded. However, an estimated 13 million units of blood worldwide are not tested against human immunodeficiency viruses or hepatitis viruses, and in some developing countries 80% of the blood supply comes from paid or replacement donors (family friends or acquaintances) even when the rate of infection in the population is high [7]. For the last 70 years since the publication of a report by Amberson et al. [8] there have been global attempts to find a genuine blood substitute. A reliable supply of safe blood is essential to improve health standards at several levels, especially among women and children, and particularly in the poorer sections of society anywhere in the world. Apart from mortality related to complications of pregnancy and childbirth among women, malnutrition, thalassaemia, and severe anemia are prevalent diseases in children which require blood transfusion, apart from other complicated diseases.

Over 80 million units of blood are collected every year, but the tragedy is that only 39% of this is collected in the developing world which contains 82% of the
global population. On the other hand, with over 100 million births globally each year, more than 10 billion milliliters of human umbilical cord blood (HUCB) are produced, the vast majority of which is totally discarded as trash. In one chapter, Dr. Niranjan Bhattacharya, Calcutta, India, discusses the transfusion perspective of freshly collected and properly screened cord blood. The author is of the opinion that the transfusion of cord blood is safe in case of anemia of any etiology, i.e., from thalassemia, HIV, leprosy, uncontrolled diabetes with albuminuria, advanced cancer, arthritis, tuberculosis, malaria and emergency condition necessitating blood transfusion support. What is interesting is the observation that there is a transient peripheral rise of CD34 in the peripheral blood without clinical graft-versus-host reaction, in HLA randomized transfusion without the support of immunosuppressive or growth factor on the host system. This hitherto unreported phenomenon could indicate a potential for immunotherapy from cells in cord blood that might improve prognosis. This observation, if verified in other institutions, may have enormous clinical significance. This phenomenon could also help in the understanding of the etiology of any advanced disease on the basis of a local or regional or selective structural or functional immunosuppression affecting different organs or its subsystem level. The question asked by Bhattacharya is whether immunosuppression is a mosaic like phenomenon. He observes that only time will prove or reject this clinical work based hypothesis (Part III, Chapter 10).

H.K. Basu, a senior consultant in Ob/Gyn, UK, has given an overview of the use of cord blood and has noted that the yield of cord blood varies from a volume of 67–134 ml mean 88 + 14 ml SD and mean hemoglobin 17.6 gm% (Part III, Chapter 13). This blood has a much higher hemoglobin (mostly fetal hemoglobin), platelet, and leukocyte content than adult whole blood. Additionally, it has a high concentration of cytokine/growth factors in its plasma, which eventually helps in the gene-switching mechanism after the birth of the baby. This blood has a much higher oxygen-carrying capacity than that of adult whole blood, and hence, the transfusion of fetal hemoglobin rich cord blood has the potential for better tissue perfusion of oxygen (vol/vol) to the recipient’s tissue than an identical volume of adult whole blood.

The futuristic potential of the use of cord blood in medicine and engineering has been touched upon by different distinguished authors, concentrating in this field of cord blood stem cells. Dr. A. Chaudhuri, a senior consultant neurologist of the Essex Neuroscience Centre (Part III, Chapter 11), UK, and Dr. Niranjan Bhattacharya from the Advanced Medical Research Center Hospital, Calcutta, India, described neurological diseases as responsible for significant disability all over the world. Based on the epidemiological data presented in the Global Burden of Disease 2000 study of the World Health Organization, it was calculated that in Europe, brain diseases account for a third of all disabilities and this is likely to increase in the coming years especially in the absence of any effective and established therapy for neuronal regeneration and repair. It is therefore imperative that potential strategies to minimize the burden of brain disease are rapidly developed and tested by ethical research and clinical trials. The authors cited many animal studies involving umbilical cord blood in brain cell repair. In an animal study, twenty-four hours after traumatic brain
injury, umbilical cord blood was administered intravenously in the rats. Treated animals showed significant improvement in the neurological deficit compared with the control animals by 4th week. In another experimental mouse model (G 93A) of motor neuron disease (amyotrophic lateral sclerosis), intravenous administration of umbilical cord blood in pre-symptomatic animals resulted in a delay of the disease progression by 2–3 weeks and increased life span in the diseased mice. The transplanted cells survived for 10–12 weeks after administration and entered in the areas of motor neuron degeneration in the brain and spinal cord where they were found to express neural markers. In addition, the transplanted cells were widely distributed in the peripheral circulation and in the spleen. High volumes of HUCB mononuclear cell infusion in a mice model of Huntington’s disease (B6CBA-TgN 62 Gpb mice) reduced the rate of weight loss, which appears before the onset of chorea, and total duration of survival. These workers also found improved survival of mice overexpressing amyloid precursor protein (a model of Alzheimer’s disease) with high dose cell therapy.

**Bioengineering Application of Cord Blood**

Cord blood stem cells also have application in the field of bioengineering. According to Dr. D. Basu and his associates, a globally renowned scientist group from India working on the ceramic biofriendly interface, the main cause of premature failure of an orthopedic implant in vivo is due to various biological reactions with the surrounding tissues/environment (Part V, Chapter 17). To combat this situation, continuous efforts have been concentrated on the improvement of the biocompatibility of the implant material by adopting different strategies. MSCs are one of the adult stem cell types that can be made to develop a limited number of different kinds of tissues, with bone, cartilage, muscle and skin, being the most important types. They are found primarily in the bone marrow, but are also available elsewhere in the body such as in umbilical blood or fatty tissue. One possible application for these versatile cells is to aid in the production of optimized materials for bone implants, such as those used in artificial hip joints.

Another internationally renowned bioengineering group from India, Dr. K. Kaladhbar, and Dr. Chandra P. Sharma have also commented on the futuristic potential use of cord blood stem cells in making a healthy long lasting biofriendly interface in case of different orthopedic and neurosurgical implants (Part V, Chapter 16). They propose that the major problem associated with medical devices implanted inside the body is performance failure due to biological reactions, regulated by the adsorbed proteins and the pathological cells on the material surface. The surface of the material is being modified to make the material biocompatible. Introducing specific surface groups, immobilizing proteins with certain conformations, or by immobilizing certain cell lines, often does this. Strategies have also been adopted to modify the material/biology interface. Basically this type of cell-mediated therapy is being done to improve the device integration by augmenting
the tissue regeneration, e.g., endothelialization of the vascular grafts to improve the blood compatibility; utilizing platelet rich plasma for improving tissue regeneration in periodontitis, etc. Cord blood stem cell, the investigators feel, could potentially be a very useful material in such diverse applications.

**Ethics and Cord Blood**

In our final chapter, Prof. Ranesh Chakraborty (Part VI), former Chairman Surgical Sciences, University of Virginia, USA, considers the ethical aspects on stem cell biology specially involving the cord blood stem cells. He notes that the English word ‘ethics’ has some equivalence with the Sanskrit term ‘dharma,’ Dharma means the principles that ‘support’ or guide an individual in the passage through life. As he states, the major ethical controversy is the source of stem cells, whether from embryonic tissue or not. Since ethical opinions on this issue vary widely in the diverse belief systems of our world, one possible solution to reducing conflict may be to explicitly identify the source of stem cells lines. As one example, this would allow those to benefit who can accept stems cells from embryos donated by infertile couples but not from embryos specifically obtained for treatment of disease.

Today, if there is any problem in medicine not amenable to conventional treatment, the application of stem cell technology may offer new hope, but we must not sacrifice our objectivity in proper assessment of the situation. In this connection, the famous teaching of the legendary scientist Prof. J.B.S. Haldane is appropriate: “Science is vastly more stimulating to the imagination than are the classics” (Daedalus) [J.B.S. Haldane, *Daedalus* (in Henry Davidoff, ed., *The Pocket Book of Quotations* (Pocket Books, New York, 1952), p.329].

**References**


