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The umbilical cord: a rich and ethical stem cell source to advance regenerative medicine

N. Forraz and C. P. McGuckin

CTI-LYON, Cell Therapy Research Institute, Parc Technologique de Lyon St Priest, St Priest-Lyon, France

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Abstract

Science and medicine place a lot of hope in the development of stem cell research and regenerative medicine. This review will define the concept of regenerative medicine and focus on an abundant stem cell source – neonatal tissues such as the umbilical cord. Umbilical cord blood has been used clinically for over 20 years as a cell source for haematopoietic stem cell transplantation. Beyond this, cord blood and umbilical cord-derived stem cells have demonstrated potential for pluripotent lineage differentiation (liver, pancreatic, neural tissues and more) *in vitro* and *in vivo*. This promising research has opened up a new era for utilization of neonatal stem cells, now used beyond haematology in clinical trials for autoimmune disorders, cerebral palsy or type I diabetes.

Introduction

'Stem cells'; never in the history of science and medicine have two words sparked off so much interest, passion, controversy and hope from the scientific, medical, public, ethical, religious, political and commercial communities. It is, however, important to state clearly that despite many significant clinical achievements and great promises, stem cells are not the sole means to cure all diseases. Biomedical research and future treatments will always rely on innovation in medicine, surgery, technology and/or pharmaceutical developments.

This review will outline concepts surrounding stem cell applications and regenerative medicine and focus particularly on a fascinating and abundant stem cell source – the umbilical cord. This tissue physiologically supports development of the child throughout foetal life until birth, and can further be used for biomedical research and clinical applications.

Correspondence: C. P. McGuckin, CTI-LYON, Cell Therapy Research Institute, Batiment Cèdre 1, 97 Allée Alexandre Borodine, Parc Technologique de Lyon-St Priest, 69800 St Priest-Lyon, France. Tel.: +33 426 03 01 30; Fax: +04 26 03 02 22; E-mail: office@conoworld.com

Regenerative medicine

The advancement of science, medicine and surgery has helped mankind improve global health, albeit with significant disparities in accessing healthcare worldwide between developed and emerging countries, but many definitions have been proposed for the term 'regenerative medicine' (1–3). Kaiser, a health economist forecasting future medical technologies, first presented this concept in 1992 as an attempt to alleviate chronic diseases and restore damaged and failing organs (4).

With the development of immunosuppressive regimens, transplantation medicine and surgery in the 20th century and now the 21st, have enabled treatment of patients who would have had no therapeutic alternatives. However, shortage of donor organs increased significantly with clinical demand.

Taking liver as an example, it is estimated that 70% of patients awaiting liver transplantation in European Union countries will never find a donor. This persistent shortage of liver donors has led to mortality rate of 20% per year from the waiting list (5).

Our modern lifestyles have also increased prevalence of diabetes (type I and II) and cardiovascular diseases, which both cause major complications (stroke, kidney failure and more), and in the USA alone account for annual health care costs as high as 174 billion dollars and 475 billion dollars, respectively (source: USA National Institute of Health).

These challenges represent opportunities for the field of regenerative medicine. This aims to gather different scientific specialties and technologies to restore impaired functions in tissues and organs that have been damaged by illnesses, accidents or even by treatments.

Innovation and research in nanotechnologies, biomaterials, tissue engineering, bio-imaging, cells and stem

cells are key to the advance of regenerative medicine, as demonstrated with recent case studies and clinical trials. For instance, in 2006, Atala and colleagues isolated cells from patients with bladder dysfunction and cultured each patient's own (autologous) cells on bioscaffolds in the shape of a bladder, in the laboratory. These artificially engineered bladders were later successfully re-implanted into the patients and restored their function (6). More recently, Macchiani and colleagues of an international consortium transplanted a young adult in Spain with a tissue engineered trachea segment. The donor trachea was made acellular and seeded with the recipient's own epithelial cells and mesenchymal stem cell-derived chondrocytes that had been cultured *in vitro* (7,8). This technique was repeated for a child using a longer trachea segment in 2010 in the United Kingdom. So far, no complications have been reported as donor tracheas were decellularized and further reconstructed using the patient's own cells. No rejection nor immune complication has been reported. At the time of writing, both patients are leading a normal life without immunosuppression (18 months and 5 months post-transplantation, respectively).

Such exciting and safe clinical cases of modern regenerative medicine illustrate the need for interdisciplinary research and understanding the full potential of stem cells for clinical applications.

Stem cells

Stem cells are defined by their capacity to divide and produce (at least one) identical stem cell (self-renewal) and for one to undergo lineage differentiation (9). Depending on potency of stem cells to produce one or more lineages, they can be identified as totipotent (for example the zygote, the only mammalian cell capable of producing all cells and tissues of an organism), pluripotent (with capacity to produce cells and tissues from all three germ layers – ectoderm, mesoderm and endoderm), multipotent (capacity to produce more than one cell lineage) or unipotent (differentiation into a single cell phenotype).

Early in the 1900s, Maximow was the first to propose that lymphocytes acted as common *stem cells* and migrated through tissues to form blood circulation components (10), but the 1960s shaped the true beginning of stem cell research as we know it today. Research by Till and McCulloch (11) and by Goodman and Hodgson (12) demonstrated in mice, that the bone marrow hosted stem cells, from which clonogenic precursors could be derived and could restore haematopoiesis in irradiated animals. This work simultaneously evolved with the advent of human stem cell transplantation for bone marrow replacement (13). At the same time, research by Edwards and colleagues generated the first embryonic stem cell lines in rabbits (14), which much later advanced into the development of the first human embryonic stem cell line (15). Stem cells today can also be categorized according to the source of tissues from which they originate.

Embryonic stem cells

Human embryonic stem cells (ESC) are derived *in vitro* from the blastocyst of an embryo usually left over from *in vitro* fertilization. ESC are cell lines derived from the embryoblast of early embryo at the blastocyst stage. They proliferate *in vitro* while maintaining an undifferentiated state, and are capable of differentiating into many specialized somatic cell types under appropriate conditions (pluripotency). Much fascination and controversy has fuelled the world of biomedical research since derivation of the first human embryonic stem cell lines, as it induces destruction of a human embryo. However, beyond ethical objections raised by research on human ESCs, significant technical hurdles have slowed their progress towards clinical application, not least their immunogenic status, spontaneous formation of teratocarcinomas upon transplantation and genetic/genomic instability in cell culture systems during scale-up (16).

Adult stem cells

Adult or somatic stem cells can be isolated from specific adult human tissues (brain, skin, gut, bone marrow, fat, cornea and more). They have limited ability to regenerate damaged tissues physiologically. Although their differentiation potency is considered to be less than ESC by some scientists, their isolation, characterization and translation to pre-clinical and clinical studies have increased during the past two decades, not least in the field of haemato/immunotherapies, but also recently for certain cardiovascular indications (17), wound healing (18,19), corneal repair (20) or even although less advanced, for multiple sclerosis (21). Beyond tissue-specific stem cells, mesenchymal stem cells (MSCs) were first characterized as a specific bone marrow-derived fibroblast-like adherent cell population with potential and capacity to support haematopoiesis (22,23). Further studies demonstrated their potential to differentiate initially into three specific lineages: osteocytic, chrondrocytic and adipose lineages and later on into many endodermal, mesodermal and ectodermal tissues (24,25).

Different adult tissues have been proposed as sources for MSCs: bone marrow, adipose tissues, synovium, dental pulp and more. Clonogenic assays and putative markers have also been proposed for MSCs, the biology of which is becoming better understood and standardized (26).

Induced pluripotent stem cells

The recent discovery of induced pluripotent stem cells (IPS), by the initial work of Yamanaka and colleagues first in mice then in humans, has circumvented to a certain extent some ethical and scientific limitations of ESC research (27). This technique consists of using somatic terminally differentiated cells and inducing expression of a number of genes therein, to produce stable lines of embryonic-like pluripotent stem cells. This technique offers the interesting possibility of creating patient-specific stem cell lines for research, and perhaps one day, diagnostic applications without the controversial use/destruction of human embryos. However, its significance for relevant clinical applications remains unknown as yet, mostly because of the low efficiency of such induced gene expression. New techniques are being investigated to generate IPS cells with minimal or no exogenous genetic modifications (28).

Neonatal stem cells

Our group previously proposed a distinct category of somatic stem cells called 'neonatal' stem cells, derived from various biological tissues often considered as biological waste after birth, rather than biological resources, namely amniotic fluid, placenta, umbilical cord and cord blood (29,30). With over 135 million births per year worldwide (source: USA Central Intelligence Agency Factbook 2009), neonatal tissues are objectively the largest and most genetically diverse stem cell source that can be accessed in a non-invasive, rapid and cost-effective manner during and after birth. This review will particularly focus on the umbilical cord as a stem cell source for biomedical research and clinical applications.

The umbilical cord as a source of stem cells

From the third week of development, the human embryo becomes attached *via* a connecting 'stalk' to the forming placenta. At week 5, a primitive umbilical cord is formed in the shape of an umbilical ring. At week 10, after development of the gastrointestinal tract in the foetus, the umbilicus appears as a hernia linking into the umbilical cord.

The umbilical cord is covered by an amniotic epithelium which protects a gelatinous and elastic matrix made of mucopolysaccharides (mostly hyaluronic acid and chondroitin sulphate) called 'Wharton's jelly' named after Dr Thomas Wharton who first described it in 1656 (31). The amnion and Wharton's jelly protect three blood vessels that are crucial for embryonic and foetal development. One large umbilical vessel supplies the developing foetus with placental blood, rich in nutrients and oxygen, and in the last trimester with important antibodies provided by the mother. Two smaller umbilical vessels return from the foetus the blood with carbon dioxide, wastes and other toxins.

The umbilical cord can provide stem cells from the blood running in the umbilical vessels, walls surrounding the vessels and from the Wharton's jelly.

Cord blood stem cells

Cord blood can be collected at birth using a sterile collection kit consisting of an anticoagulant (usually citrate or heparin)-containing collection bag connected to one or several collecting needle(s). Cord blood samples can be collected *in utero*, after the birth of the child and before delivery of the placenta or *ex utero*, from normal deliveries and caesarean sections with no pain for the mother or the child (Fig. 1).

In a recent study, our group demonstrated that cord blood stem cells and other cell populations, in general, were influenced by obstetric history and other maternal factors (30). Cord blood units are usually transferred to a laboratory where they undergo cell separation to extract the buffy coat and/or cell preparations enriched with stem cells. Different techniques exist to extract cells from cord blood, such as: centrifugal elutriation, rouleaux formation, starch-based methods, and density-gradient methods, among others (32–34) (Fig. 1).

Historically, umbilical cord blood has been known to contain haematopoietic stem/progenitor cells with that have the ability to produce clonogenic progeny. In 1974, Knuddtzon (35) was the first to confirm presence of cells with haematopoietic clonogenic potential in cord blood *in vitro*. Broxmeyer and colleagues published in 1989 a report confirming presence of haematopoietic stem cells in cord blood (36). Further studies verified their clonogenic potential, self-renewal property and capacity to be expanded *in vitro* (37–41).

Our research group and others spent many years analysing the different cell groups present in cord blood to distinguish between stem cell and progenitor cell phenotypes, and later on between haematopoietic and non-haematopoietic cell groups (40,42). In 2004, our team reported for the first time, the discovery of non-haematopoietic pluripotent embryonic-like stem cells from cord blood, named cord blood embryonic-like stem cells (CBEs). Further investigation of these cells demonstrated that they could be harvested from fresh and cryopreserved units and had expansion and differentiation potential into neural, hepatobiliary and pancreatic-like precursors (43–50). This ground-breaking discovery, albeit challenged at the time, has since been confirmed by several other groups (51–55).

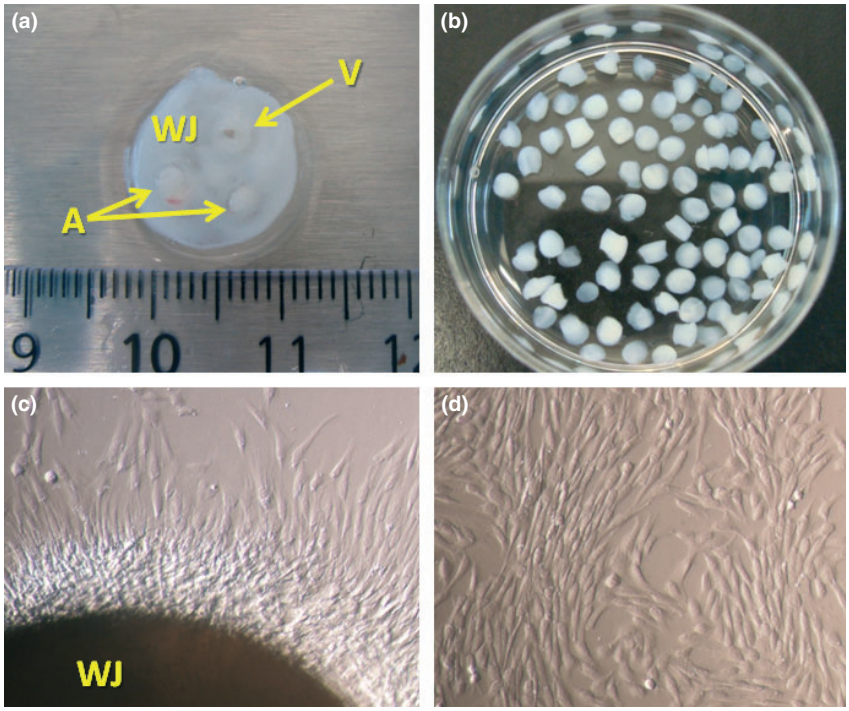

Figure 1. Umbilical cord Wharton's jelly as a source of mesenchymal stem cells. (a) Sagittal section of a 1 cm diameter umbilical cord with Wharton's jelly (WJ) surrounding two arteries (A) and one vein (V). (b) 10 mm^3 biopsy pieces of Wharton's jelly. (c) Wharton's jelly piece (WJ) in serum-free culture growing-out mesenchymal stem cells. (d) Mesenchymal stem cells from umbilical cord at 80% confluence in serum-free culture.

Further to this, our group and others have also been able to identify and isolate multipotent MSCs from cord blood with more restricted differentiation potential and significant variability between samples (unpublished observations; 56–58).

These studies have demonstrated that cord blood has potential beyond haematopoietic differentiation and could be considered for further regenerative medicine research (59).

Umbilical cord Wharton's jelly stem cells

Several groups have recently reported the possibility of deriving MSCs, not only from cord blood but also from the umbilical cord matrix – Wharton's jelly. McElreavey and colleagues first reported in 1991 the possibility of isolating fibroblast-like cells with population growth potential from the Wharton's jelly (60). Several techniques have been reported to dissect Wharton's jelly mechanically and/or digest it enzymatically to culture homogeneous MSC populations.

The French Academy of Medicine presented a report in January 2010 in which they considered that research on umbilical cord stem cells was extremely promising and could provide useful new tools for treatment of several diseases. A single piece of 5–10 mm^3 Wharton's jelly has the potential to yield as many as 1 billion MSCs in 30 days (Degoul O., Jurga M., Forraz N. and McGuckin C.P. unpublished personal data). With the average umbilical cord measuring 50 cm, one could predict that this source of MSCs will become more and more clinically relevant as research advances (Fig. 2). Umbilical cord Whar-

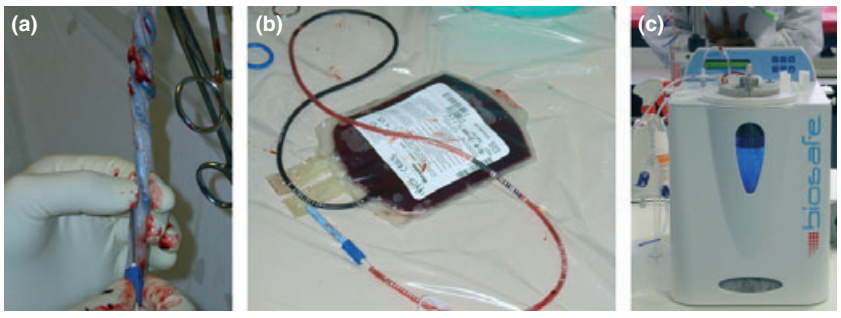

Figure 2. Umbilical cord blood collection and processing. (a) Cord blood is collected after birth from the umbilical vein into (b) citrate-based anticoagulant-containing blood collection bag. (c) Sepax device, enabling closed system cord blood processing in approximately 20 min.

ton's jelly-derived MSCs are increasingly being considered as more robust than those from cord blood itself and, by nature, they are less invasive than those from the bone marrow (61). Several studies have shown that umbilical cord-derived MSCs can be differentiated into bone (62,63), skin (64), endothelium (65), hepatocyte (66,67) and neural lineages (68) to name but a few. The immunomodulatory properties of umbilical cord MSCs were shown to be similar to bone marrow-derived MSCs (69). The potential of this stem cell source is therefore enormous for regenerative medicine applications.

Umbilical cord blood vessels

The umbilical cord is well known as a source of endothelial progenitor cells. These have been identified for angiogenesis and vasculogenesis research and as model tissues, not least with the now standard isolation of human umbilical cord vein endothelial cells (HUVEC) (70). Their role in haematopoiesis has also been demonstrated as HUVECs produce growth factors and adhesion molecules that can induce maintenance and proliferation of cord blood haematopoietic progenitors (71). In 2003, Saraguser and colleagues proposed that the umbilical vein and HUVECs were a source of perivascular cells – pericytes. For their study, they hypothesized that umbilical blood vessels were a potential source for a distinct population of pericytes, which were ancestors of MSCs found in Wharton's jelly (72). This hypothesis has since been corroborated in supplementary studies, which further identified these pre-MSC pericytes in other adult tissues (73,74).

Umbilical cord and cord blood stem cells for regenerative medicine

Haemato/immunotherapies

As early as 1939, Dr J Halbrecht, Beilinson Hospital, Judah and Sharon regions, Israel, published two reports on the use of placental (umbilical cord) blood for transfusion purposes. Placental blood stored for up to 15 days was used for 220 transfusions with minimal or no undesired effects observed in patients (75,76). Ende and Ende reported in 1972 the first clinical case (in 1970) using eight umbilical cord blood units to transfuse a 16-year-old patient suffering from leukaemia (77). These studies led to transplantation into a child suffering from the bone marrow disorder, Fanconi's anaemia, with his sister's umbilical cord blood, in 1988; her cord blood sample was collected and conditioned at birth (78). Wagner and colleagues in Minnesota, USA initiated the first volunteer family banks for young patients who had a compatible sibling from which cord blood could be collected at birth, in 1995 (79). Technical progress in bone marrow transplantation was hindered, however, by the shortage of suitable HLA-compatible bone marrow donors, and in 1991, the New York Blood Center created the first public cord blood bank in the USA. This today holds the largest cord blood public registry and in 1995 provided a cord blood sample for the first unrelated cord blood transplant (80). Cord blood compared to bone marrow, was rapidly identified as a valuable stem cell source for clinical applications; it offers more tolerant HLA matching between donor and recipient, results in less graft-versus-host disease and is readily available from biobanks. In bone marrow transplantation though, time for engraftment and cell dose available have been highlighted as major drawbacks of using cord blood as an option for haematological transplantation. However, since the mid-2000s double and triple cord blood unit transplants have been standardized and applied to children and adult patients. Recent findings on graft engineering and robust *ex vivo* expansion protocols have also increased the potential of cord blood for immune and haematopoietic reconstitution (81,82). Today, over 20 000 cord blood transplantations have taken place worldwide for related (sibling) and unrelated allogeneic transplantation, mostly to treat patients with haematological and immunological conditions needing to restore haematopoiesis or their immune systems (83). Very few clinical cases have been reported of autologous use of cord blood for transplantation to restore haematopoiesis (84–86).

Umbilical cord and cord blood stem cells for non-haematopoietic applications

Advances in biomedical research and thorough regulatory environments will jointly contribute to development of clinical trials for stem cell-based therapy. Scientists and clinicians should not have a haematocentric perspective for use of cord blood and umbilical cord cells for regenerative medicine, as recent clinical studies widen the potential of neonatal stem cells for clinical applications beyond haematotherapies.

Type 1 diabetes. Type 1 diabetes is an autoimmune disease that causes destruction of insulin-producing pancreatic beta cells, by T cells. This disease is managed by patients in lifelong administration of exogenous insulin. Following several *in vitro* and *in vivo* animal studies, a prospective clinical trial has taken place under the supervision of Dr MJ Haller and colleagues at the University of Florida, USA, in which 15 young children received infusions of their own umbilical cord blood cells. Three to six months post-infusion, all patients demonstrated slowing of loss of endogenous insulin production correlated with lowered daily insulin requirements, improved HbA1c lev-

els and increase in regulatory T cells, suggesting potential immune-modulatory effect as a mechanism of action for this treatment. Although 1-year post-infusion assessment confirmed the safety of autologous cord blood therapy for type I diabetes, no significant improvement in C-peptide endogenous production, insulin requirements or HbA1c levels were observed by this time. Further to this, no changes in T-cell phenotype ratios nor autoantibody titres were seen. It was further suggested that cell dose and multiple time-lapsed infusions of cord blood cells could be necessary to improve glycaemic regulation in type I diabetes patients (87–89).

Systemic lupus erythematosus. Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder caused by production of autoantibodies against connective tissues, leading most usually to significant inflammation of skin, joints and kidneys, although other organs can also be affected. At the University Medical School of Nanjing, China, a single arm safety and efficacy clinical trial on 16 SLE patients refractory to standard therapy, on reception of allogeneic umbilical cord-derived MSC transplantation, has recently been reported. Fifteen months post-transplantation, all patients experienced significant amelioration of disease (recorded by SLE disease activity index) and renal function, improved serological results (antinuclear antibody, anti-double-stranded DNA antibody, C3 complement, albumin), increase in regulatory T cells and stabilization of pro-inflammatory cytokines. These initial encouraging results will be followed by a randomized controlled clinical trial (90).

Epidermolysis bullosa. Epidermolysis bullosa (EB) is an inherited mutational disorder, causing skin to be deficient in collagen, laminin, integrin and/or plakin. Patients usually suffer from severe blistering of the skin and mucosal membranes. A prospective clinical study being carried out by Pr Wagner and colleagues at the University of Minnesota Medical Centre has concerned patients undergoing bone marrow and umbilical cord blood stem cell transplantation. Preliminary results from this study have shown that patients experienced correction of the disease with reduction in blistering and production of healthy skin (91 and communication at the Responsible Stem Cell Research Conference, November 2009, Monaco and clinicaltrials.gov ref# NCT01033552).

Inherited metabolic diseases. Many patients with inherited metabolic diseases experience progressive degeneration of the central nervous system. Cord blood stem cell transplantation (as well as bone marrow and peripheral blood-derived stem cells) has already been used to treat patients with inherited metabolic diseases with lysosomal and/or peroxisomal storage disorders. Several pre-clinical and clinical studies have confirmed that cord blood-derived cells containing normal levels of enzymes have high potential to migrate to non-haematopoietic organs and trigger cross-correction of the recipient's enzyme-deficient cells and may account for a certain degree of neural regeneration (92).

Neonatal asphyxia and cerebral palsy. Cerebral palsy is a generic term referring to a number of disorders that appear in early childhood, affecting muscle coordination and body movement. Foetal, neonatal and post-birth asphyxia often lead to cerebral palsy disorders due to neurological lesions incurred.

Beyond neurological improvement observed in patients treated for inherited metabolic diseases, several pre-clinical studies have demonstrated that human cord blood-derived stem cells could induce endogenous neural repair processes. Although the precise mechanisms of action remain to be confirmed, infusion of cord blood cells following brain ischaemia has been shown to induce neurogenesis, and to bring trophic factors with neuroprotective effects to sites of injury (29,30,59).

Further, a pilot non-randomized clinical study at Duke University, USA, is currently assessing safety of autologous umbilical cord blood cells in newborn infants with hypoxic–ischaemic encephalopathy. Cord blood cells were provided by 14 private biobanks and to date 188 patients (aged 1 week–9 years) have been infused with autologous cord blood cells with a minimum cell dose of 1×10^7 cells/kg. Infusions were well tolerated and no clinical adverse effects have yet been reported. These children will be followed up for neurodevelopmental outcome and functional MRI imaging (clinicaltrials.gov ref# NCT00593242 and Kurtzberg in communication at 8th Annual International Transplantation Symposium, San Francisco June 2010).

In an additional study, a randomized observer-blinded crossover clinical trial, has just been initiated at the Medical College of Georgia for children diagnosed with cerebral palsy, whose parents had saved their infants' cord bloods. Patients to be included are aged between 2 and 12 years and have clinical evidence of a non-progressive motor disability (clinicaltrials.gov ref# NCT01072370).

Storing neonatal stem cells

Much scientific, ethical and political debate surrounds the concept of storing umbilical cord blood and other neonatal cells and tissues, for clinical applications. In many countries parents receive information consent to donate umbilical cord blood to a public bank where samples are anonymized and stored for future unrelated (or sometimes

related-sibling) allogeneic purposes (generally only for haematotherapy) as long as they meet certain quality control criteria. Costs of storage are supported by public banks, usually through public state funding. Further parents decide to store umbilical cord blood with private biobanks through a payable service over several years or decades. Cord blood samples are then available for future autologous or sibling-related allogeneic use, if required. Recent initiatives however, have offered a third model for storage of umbilical cord blood, through a mixed banking model whereby samples are stored by the family but can also be donated to suitable patient/s needing transplantation.

To date, only just over 400 000 cord blood samples are available from public registries worldwide and we estimate that a further million samples are stored in private biobanks (Fig. 3). Private and public biobanks have also recently begun to offer storage of the umbilical cord as a potential source for autologous and allogeneic regenerative medicine applications. We strongly believe that most parents should be informed of storage options regarding umbilical cord blood or other neonatal cells and tissues, as this extraordinary bioresource is widely available worldwide in developed and emerging countries. Furthermore, advancement of regenerative medicine now requires that other medical disciplines have both opinion and an interest in uses of umbilical cord and cord blood-derived cells. No longer must we allow haematologists, who are not experts in other medical disciplines, to prevent other clinics from developing and using these important stem cell sources. In our recent landmark paper comparing different ways to separate cord blood stem cells of clinical grade, we have shown that not all current methods are appropriate for regenerative medicine (34). In the future, there is a need to have a variety of banks, where cord blood and placental tissues are processed using a range of methods, then made available to a wider group of clinicians.

Conclusion

Although many types of stem cells have been proposed in recent years, much hyperbole unfortunately exists. In reality, umbilical cord and cord blood-derived stem cells remain the world's largest potential source, bearing in mind the global birth rate of around 135 million per year. The exponential rise of cord blood banking also shows the global interest in the use and need for these cells. Through regenerative medicine, we and others have proven that these placental- and umbilical-derived tissues, which would otherwise be thrown away, must be considered for use either immediately or after storage. With no ethical controversies in collection of these umbilical cord stem cells, the only question that remains is the potential for defined clinical trials. For this, governments need to be ready with cell therapy legislation to allow cells' rapid transit to hospital clinics, while ensuring patient safety.

Acknowledgement

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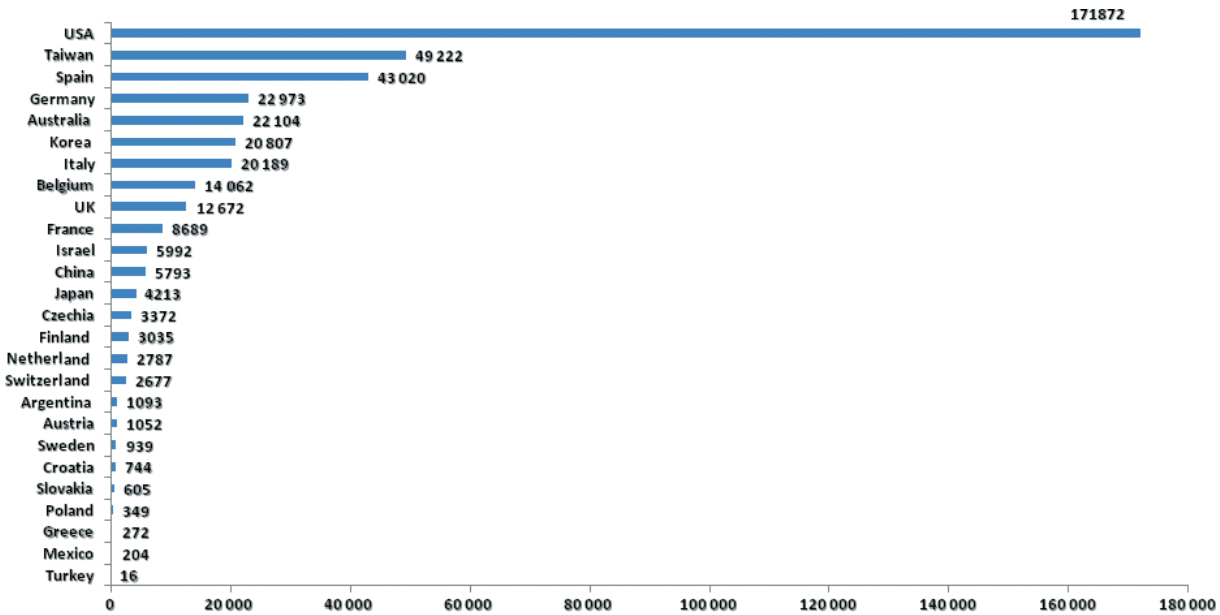

Figure 3. Over 419 000 umbilical cord blood samples are stored in public registries worldwide (source Bone Marrow Donor Worldwide http://www.bmdw.org, March 2010).

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