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Cell- & Tissue-based Therapy

The potential of cord blood stem cells for use in regenerative medicine

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It is estimated that up to 128 million individuals might benefit from regenerative medicine therapy, or almost 1 in 3 individuals in the US. If accurate, the need to relieve suffering and reduce healthcare costs is an enormous motivator to rapidly bring stem cell therapies to the clinic. Unfortunately, embryonic stem (ES) cell therapies are limited at present by ethical and political constraints and, most importantly, by significant biologic hurdles. Thus, for the foreseeable future, the march of regenerative medicine to the clinic will depend on the development of non-ES cell therapies. At present, non-ES cells easily available in large numbers can be found in the bone marrow, adipose tissue and umbilical cord blood (CB). Each of these stem cells is being used to treat a variety of diseases. This review shows that CB contains multiple populations of pluripotent stem cells, and can be considered the best alternative to ES cells. CB stem cells are capable of giving rise to hematopoietic, epithelial, endothelial and neural tissues both *in vitro* and *in vivo*. Thus, CB stem cells are amenable to treat a wide variety of diseases including cardiovascular, ophthalmic, orthopedic, neurologic and endocrine diseases.

Keywords: arthritis, brain, cartilage, cornea, diabetes, heart, liver, regenerative medicine, spinal cord injury, stem cell, stroke, umbilical cord blood, wound healing

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1. Introduction

As a prelude to this review, let it be stated that one cannot hope to cover all works and authors in this field of study even when the review is limited to the topic of cord blood (CB) stem cells and regenerative medicine; thus, my apologies to anyone who has been overlooked, it was not intentional. Further, it is also possible that some of the most recent work may not be mentioned due to time constraints in the preparation of this review; again, my apologies.

Over the last five years, the field of regenerative medicine has exploded. It is estimated that as many as 1 in 3 individuals could benefit over their lifetime from the applications of regenerative medicine, including therapies for cardiovascular disease, endocrine and orthopedic treatments. Scientific and medical researchers have published a wealth of data to support the use of stem cells to treat a range of common, previously untreatable diseases, including diabetes, myocardial infarction, stroke and spinal cord injury. Although the political and ethical controversy surrounding the use of embryonic stem (ES) cells has cast an unnecessary negative shadow over these numerous breakthroughs, growing public awareness of umbilical CB as an alternative stem cell source has heightened the enthusiasm and demand for this area of research.

The recent emergence of regenerative applications for CB stem cells are based to some extent on their use in treating a range of hematologic malignancies and blood disorders such as leukemia, lymphoma and sickle cell anemia. This clinical experience over the past 15 years has led to some of the findings that CB stem cells can give rise to tissues other than blood and immune cells.

2. Cord blood is an established bone marrow equivalent

CB stem cells have long been touted as a rich source of hematopoietic, or blood-forming, stem cells for transplantation. As of 2007, > 8000 CB transplants have been performed worldwide and numerous public and private agencies have emerged to store CB for public or familial use. The public CB banks are similar to bone marrow registries with the exception that the cells are cryopreserved and readily available for transplantation. Stem cell banking eliminates the danger of donor attrition. In addition to being readily available, CB has other distinct advantages over bone marrow and peripheral blood stem cells for hematopoietic transplantation, including greater tolerance for human leukocyte antigen mismatches between donor and recipient and decreased graft-versus-host disease. However, regenerating blood and bone marrow is merely the tip of the iceberg; as extensive research has shown that CB stem cells have the ability to regenerate numerous tissue types and, when transplanted into animals and humans, have produced measurable functional improvements (to be discussed below). The number of tissue types that CB stem cells regenerate can be attributed to the presence of several cellular populations with varying degrees of pluripotential ability. These populations of stem cells within CB include hematopoietic, endothelial, epithelial, mesenchymal and, most significantly, a population of embryonic-like, stem cells.

3. Cord blood contains pluripotent embryonic-like stem cells

Although CB stem cells were shown to differentiate into numerous cell types *in vitro*, and to repair damaged neurologic, cardiovascular and hepatic tissues, the mechanism of action was poorly understood. However, in 2005, a landmark paper by Dr Colin McGuckin and co-workers demonstrated that primitive, embryonic-like stem cells could be isolated from human CB. This study represented the culmination of years of research by other investigators, each of whom were able to isolate and expand different types of progenitor cells from CB, including the building blocks of heart, nerve, bone and liver tissue. McGuckin and co-workers identified the most primitive cells to date, and were able to expand these cells up to 258-fold and to drive their differentiation into hepatic progenitor and pancreatic islet cells. They concluded that these cells had the ability to differentiate into many different cell types, which helps explain the broad therapeutic efficacy of CB stem cells [1].

A previous study had reported the identification and isolation of pluripotent, unrestricted somatic stem cells (USSCs) from human CB. These cells were negative for the $CD34^+$ cell surface marker that is most commonly used to identify hematopoietic stem cells in CB. The USSC could be expanded *in vitro* to 10^{15} cells without loss of pluripotency. When cultured *in vitro*, USSCs differentiated into osteoblasts, chondroblasts, adipocytes and neural cells [2], which putatively identifies USSCs as a novel population of mesenchymal stem cells (MSC).

CB has also been found to contain typical MSC, which give rise to many different tissue types, including osteoblasts, chondrocytes, myocytes, adipocytes, neuronal cells and insulin-producing islet cells [3-5]. These cell types include several populations with broad therapeutic implications, particularly islet cells, which could be used to treat diabetes.

Finally, CB stem cells are unique in that other than ES cells, these neonatal stem cells are the only ones to date capable of forming a complete human immune system when transplanted into mice; giving rise to B, T and dendritic cells, the formation of primary and secondary lymphoid organs and the production of functional human immune responses. This finding demonstrates that CB stem cells are distinct from typical adult stem cells [6].

Thus, CB contains a mixture of different types of stem cells in numbers not seen in any other location: embryonic like stem cells, hematopoietic stem cells, endothelial stem cells, epithelial stem cells, MSC and USSC. Cumulatively, the identification and isolation of populations of pluripotent stem cells within CB represents a scientific breakthrough that could potentially impact every field of medicine. As the properties of these cell types are delineated and understood, scientists will be better able to control their behavior both *in vitro* and *in vivo*. CB represents a novel source of stem cells positioned closer to ES cells (developmentally) than typical adult stem cells.

It should be noted that hematopoietic ($CD34^+$) and endothelial ($CD133^+$) stem cells are the most prevalent stem cell populations found in CB. USSC, MSC and embryonic-like stem cells are found in smaller numbers and potentially may require *ex vivo* expansion prior to clinical use. However, it may be possible to take advantage of clues derived from the *in vivo* milieu that would permit *in situ* stem cell expansion and differentiation by direct delivery of the mixed cell population to the site requiring stem cell therapy. Ongoing clinical trials (see below) should help determine which method is necessary.

4. Cord blood stem cells produce measurable improvement in animal models and patients with common human diseases

For years, scientists have demonstrated that CB stem cells can not only differentiate into many cell types, but can also engraft

and improve function in animal models of disease. This research is paving the way for existing and future clinical trials in human patients. The body of data are extensive for many disease states, including the following examples.

4.1 Cardiovascular disease

There is an urgent need for novel therapies for heart failure. Cardiovascular disease is the leading cause of morbidity and mortality for both men and women in the US. Approximately 1 million people die of cardiovascular disease annually despite medical intervention. Coronary artery disease comprises ~ 50% of these deaths. As heart cells have a limited capacity to regenerate after myocardial infarction (MI), application of exogenous stem cells seems a logical alternative for therapy. Recently, numerous preclinical and clinical studies have examined this question (see [7] for details and additional references). To date, only non-ES cells have been examined in clinical trials due to political, ethical and biologic constraints. Although no clinical trials using CB stem cells for heart failure have been conducted to date, a number of preclinical animal studies have been performed. Several common observations have been noted in these studies regardless of the protocols used, including selective migration of the CB stem cells to the injured cardiac tissue, increased capillary density at the site of injury, decreased infarct size, improved heart function and a lack of myogenesis. These observations are thought to be due to the production of angiogenic factors and induction of angiogenesis/vasculogenesis [8-10]. In fact, work done by Gaballa and co-workers [7,10] in myocardial infracted rats showed that $CD34^+$ CB stem cells induced blood vessel formation, reduced infarct size and restored heart function. Although no transdifferentiation was observed, the effects were thought to be due to the release of angiogenic and growth factors (e.g., VEGF, EGF and angiopoietin 1,2) induced by hypoxia as shown by gene array analyses (see **Table 1**).

Beginning in 2004, numerous studies have demonstrated in both murine and rat models of acute MI that transplantation of CB stem cells after the MI significantly improved cardiac function compared with control animals. A variety of CB populations have been used including mononuclear cell fractions, $CD133^+$ and $CD34^+$ progenitor cells, as well as $CD34^+KDR^+$ subpopulations with significant beneficial effects [10-16]. Undoubtedly, most if not all of the beneficial effects could be attributed to the induction of angiogenesis due to the release of a variety of angiogenic and growth factors [10,13] as *in vitro* work has demonstrated the CB stem cells could be induced to become/differentiate into endothelial-like cells. Although *in vitro* work has demonstrated that CB stem cells can become cardiomyocyte-like cells (as assessed by the expression of cardiac muscle troponin I and myosin ventricular heavy chain molecules), this event has been rarely observed *in vivo* [10] (and references in [7]). Interestingly, as a prelude to human clinical trials for MI, it has been shown that it is possible to isolate therapeutic cells from CB using clinical grade apparatus making the transition from bench to beside a bit more facile. Finally, work from numerous groups seems to indicate that more than one population of pluripotent cells contained in CB is capable of mediating this effect as shown by the ability of $CD34^+$, $CD133^+$ and $CD45^-$ cells to induce cardiac repair after MI [10,11,15-17]. Even more important, the numbers and potency of these cells found in CB seem sufficient for adult human applications as shown by work performed in a porcine model [15].

Aside from its application to MI, CB stem cells via the exertion of angiogenic capability also appear to be useful for the treatment of various ischemic diseases. Many investigators have demonstrated that not only does CB contain cells displaying the phenotypic characteristics of endothelial precursors that are responsible for blood vessel formation but that these cells are capable of differentiating into endothelial cells and becoming blood vessels [18-25]. These bioengineered blood vessels appeared similar to native blood vessels in terms of their (three-layered) tissue organization as well as expression of matrix components [18,20-21,23]. Furthermore, when placed in animal models CB stem cells were able to significantly reverse the effects of ischemia in several model systems [19,22,25]. In models of hind limb ischemia, transplantation of CB stem cells or endothelial cells derived from CB stem cells appeared able to reverse surgery-induced ischemia resulting in limb salvage [26-29]. Interestingly, this beneficial effect seemed to be augmented by the presence of a fibrin matrix and was equivalent to that seen with bone marrow cells in terms of capillary formation [27,28].

As before, there appears to be multiple progenitor cell populations capable of mediating these beneficial effects in ischemia as shown by a study from Schmidt *et al.* [30] in which MSC derived from the Wharton's jelly of the umbilical cord itself were shown to differentiate into mature, layered heart valve leaflets with functional endothelia. These CB-derived heart valves appeared similar to native heart valves and raise the possibility of replacing current allogeneic fixed or frozen valve products for the 275,000 patients treated annually in the US with autologous valves that provide better and longer-lasting outcomes.

To date, there have been no clinical trials using CB stem cells for cardiovascular disease. However, that fact is about to change. In great part the lack of clinical trials has been due to the relative youth of the CB banking industry. However, similar bioproducts derived from bone marrow or blood has shown promise in clinical trials. Along these lines, Osiris, Inc. [101] has an ongoing clinical trial using bone marrow-derived MSC injected intravenously to treat patients with MI. To date, a 25% increase in heart pumping function has been observed at 3 and 6 months post-treatment. Further, a recent report has been published [31] demonstrating that $CD133^+$ endothelial precursors isolated from peripheral blood (similar to those found in CB) could be used in patients to treat lower limb ischemia. In fact, 17 months postinjection in a 74-year-old patient there was limb (leg) salvage and functional improvement after this type

Table 1. Hemodynamics parameters in rats 21 days after ischemia/reperfusion.

	MAP (mmHg)	LVEDP (mmHg)	LVDP/dt (mmHg/s)	LVDP (mmHg)
Sham	115 ± 3	2 ± 1	7325 ± 98	199 ± 15
MI plus media only	102 ± 16	15 ± 3*	5725 ± 1250*	149 ± 11*
MI plus MNCs	108 ± 11	10 ± 3‡	5413 ± 1370	168 ± 12‡
MI plus CD34	99 ± 12	9 ± 4‡	5159 ± 1460	172 ± 14‡

Nude rats were subjected to myocardial infarction resultant from transient ischemia. Either CB MNC or CD34+ stem cells were injected into the coronary circulation thereafter.

*$p < 0.05$ compared to sham.

‡$p < 0.05$ compared media only.

n = 11.

CB: Cord blood; LVdP/dt: Left ventricular pressure time rate of change; LVDP: Left ventricular developed pressure; LVEDP: Left ventricular end-diastolic pressure; MAP: Mean arterial pressure; MI: Myocardial infarction; MNC: Mononuclear cell.

of therapeutic intervention. Similar observations have led to the recent announcement that similar trials using CB stem cells would begin in late 2007 at the Indiana University Medical School (Medistem, Inc. [102]) and at Duke University (Aldagen, Inc. [103]) for patients with peripheral vascular disease (for more information, please see respective websites of these companies).

4.2 Diabetes

Type 1 diabetes mellitus (T1DM) can be expected to affect 1 in every 300 births. Of the 17 million individuals with diabetes in the US, ~ 10% will display the Type 1 diabetic phenotype. Thus, ~ 2 million individuals in the US have T1DM at present. Due to the obesity epidemic now facing the country the numbers of those individuals with Type 2 diabetes (T2DM) is expected to increase significantly, especially amongst children and teenagers. T2DM is initially due to insulin resistance making cells unresponsive to the effects of insulin (although eventually presenting with insulin insufficiency), whereas T1DM is due to destruction of the β-cells in the pancreatic islets responsible for insulin production. The end result for both types of diabetes is the same: uncontrolled blood glucose. Diabetic complications include cardiomyopathy and coronary artery disease, peripheral vascular disease and neurologic complications. In an effort to treat T1DM, surgical procedures have been developed to transplant islets across histocompatability barriers with limited success due to immune rejection and the lack of donors. Thus, novel approaches are desperately needed to address this serious health issue which is thought to be responsible for 1 in every 7 dollars spent on healthcare in the US. Investigators have tried to address the issue of T1DM through the use of stem cells and regenerative medicine [32]. At present, autologous CB stem cells are being evaluated in a clinical trial to treat T1DM in children [104]. To date, 10 children have been treated, and the first child treated under the study protocol showed significant improvement in glucose control and was able to produce insulin much longer than children with a similar prognosis [33]. The protocol for the clinical trial was established in studies which showed that in animals with T1DM, those treated with CB stem cells had lower blood glucose levels, reduced insulitis, and increased lifespan compared with control diabetic animals [34,35]. This finding was confirmed in animals with T2DM as well [36]. Those animals treated with CB stem cell infusion had lower glucose levels, an increased survival curve and less kidney dysfunction as compared with control animals [36]. Similar stem cell trials are being proposed at other centers as well [104].

Although the mechanisms of action for CB stem cell therapy for either T1DM or T2DM are not known, at least in T1DM it is postulated that the infused CB stem cells once *in vivo* differentiate into new islet cells and mediate an immune tolerance to the new derived islet cells [33] (M Haller, pers. commun. University of Florida). In fact, recent results have indicated that *in vitro* CB stem cells can indeed be driven to become insulin-secreting islet cells as indicated by the production of C-peptide, an offshoot of the *de novo* secretion of insulin [37] (C McGuckin pers. commun.). In both instances, the islet cell differentiation was attributed to the presence of the embryonic-like stem cells found in CB.

4.3 Neurologic damage

Neurologic repair has a much more complex etiology than many other conditions being evaluated for CB-based therapy yet, in animal models of stroke, amyotropic lateral sclerosis (ALS), Parkinson's disease, cerebral palsy and spinal cord injury, CB stem cell infusion has resulted in observable behavioral improvement compared with control animals [38-58]. The same beneficial effect has also been observed for animals with neurologic injury due to traumatic brain injury.

Several investigators have demonstrated that it was possible to derive neurologic-like cells using CB stem cells *in vitro*. McGuckin *et al.* [38] demonstrated that ES-like CB stem cells could be expanded for up to eight weeks in culture, and that the cells expressed a neuronal cell morphology as well as neuronal markers (glial fibrillary acidic protein [GFAP], nestin, musashi-1 and nectin). These neuronal-like cells also released glial-derived neurotrophic factor (GDNF) into the cultures.

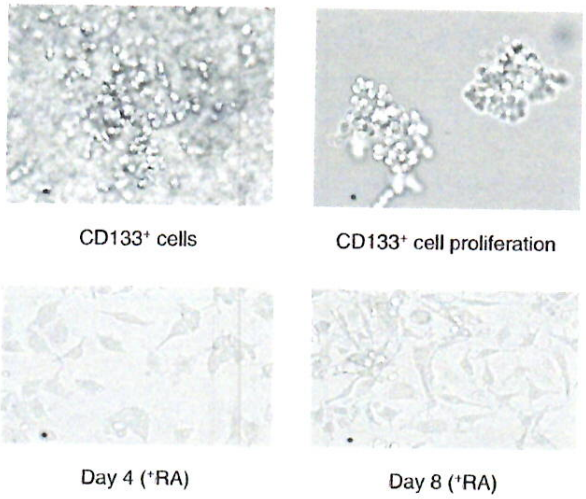

Figure 1. Differentiation of CB stem cells into neuronal cells in culture.

CD133+ CB cells were cultured as indicated in methylcellulose. Cells were evaluated at days 4 and 8 of culture with retinoic acid. Immunostaining demonstrated the presence of glial cells, astrocytes and oligodendrocytes (expression of NF, GFAP and CNPase).

CB: Cord blood; CNPase: 2,3-Cyclic nucleotide-3'-phosphodiesterase; NF: Neurofilament. RA: Retinoic acid.

Jang *et al.* [39] also showed that CB CD133+ stem cells on exposure to retinoic acid differentiated into neuronal (astrocytes and oligodendrocytes) and glial cells with neuronal markers (including tubulin βIII, neuron-specific enolase, NeuN, microtubule-associated protein-2 [MAP2] and the astrocyte-specific marker GFAP). Further, non-hematopoietic stem cells found in CB (most likely MSC) also could become neural-like cells in culture capable of becoming astrocytes and oligodendrocytes [40]. The use of these progenitor cell populations for use in cell-based treatments of brain injuries and neurologic diseases has recently been reviewed by Chen *et al.* [41]. In confirmation of these reports, work from Harris and Ahmad [42] has also shown that CD133+ and Lin- populations isolated from CB could become glial cells, astrocytes and oligodendrocytes *in vitro*. As shown in Figure 1, CB stem cells grown in methylcellulose cultures in the presence of retinoic acid for up to 8 weeks displayed a neuronal morphology and expressed cell surface markers characteristic of neuronal tissues (neuroglial cells, astrocytes and oligodendrocytes).

Nowhere has the potential significance of CB stem cell therapy for the treatment of neurologic disease been greater than in the area of stroke therapy. More than a quarter of a million individuals suffer from stroke annually in the US, often with debilitating consequences. As early as 2001, it was demonstrated that the infusion of CB stem cells into rats with the commonly used medial carotid artery occlusion (MCAO) model of stoke could ameliorate many of the physical and behavioral deficits associated with this disease [43]. Studies demonstrated that direct injection of the stem cells into the brain was not required [44] and, in fact, beneficial effects could be observed even if the stem cells did not actually make their way into the target organ (probably via the release of growth and repair factors triggered by anoxia) [45,46]. The beneficial effects seemed to be dose-dependent and could reduce the size of the infarcted tissue [47]. Once again, it appeared that multiple progenitor populations may be capable of mediating these effects [48]. Significantly, unlike existing pharmacologic interventions that require treatment in the first few hours after stroke, CB stem cell therapies were still effective up to 48 h after the thrombotic event [49]. Early studies have also shown benefit in animal models of hemorrhagic (as opposed to embolic) stroke [50]. For additional information one is referred to the recent review on cell therapies for stroke found in reference [51].

The finding that CB stem cells had the ability to become different types of nervous cells extends to other areas of neurologic damage, including spinal cord injury. Spinal cord injured rats infused with CB stem cells showed significant improvements 5 days post-treatment compared with untreated animals. The CB stem cells were observed at the site of injured nervous tissue but not at uninjured regions of the spinal cord [52]. This finding is supported by studies showing that CB stem cells transplanted into spinal cord injured animals differentiated into various neural cells, thereby improving axonal regeneration and motor function [53]. Significantly, in a recently reported clinical use of CB stem cells to treat a patient with a spinal cord injury [54] it was stated that transplantation of CB cells improved her sensory perception and mobility in the hip and thigh regions. Both CT and MRI studies revealed regeneration of the spinal cord at the injury site. Since the CB stem cells were allogeneic in origin it will be significant to see first, if the results are reproducible, and second, to determine if immune rejection or other immune-mediated problems occur that might jeopardize the improvement.

As the transfusion of CB stem cells in animals with stroke had such significant effects, it should not be surprising that similar therapies would be effective in treating other forms of neurologic damage. Along those lines, Lu *et al.* [55] have demonstrated that intravenous administration of CB could be used to treat traumatic brain injury in a rat model. In this model the CB cells were observed to enter the brain, selectively migrate to the damaged region of the brain, express neural markers, and reduce the neurologic damage. Similarly, CB stem cell transplant could also alleviate symptoms of newborn cerebral palsy in a rat model, with improved neurologic effects [56]. These observations have now been turned into clinical therapies as described below. Other investigators have shown that CB stem cells are effective therapies for Parkinson's disease [57] and ALS [58]. In both instances in the animal models CB stem cell infusion delayed symptom onset and progression, as well as prolonged survival.

Finally, CB infusions have started to make their way into the clinic to treat patients with neurologic damage. The Cord Blood Registry has released six CB stem cell samples for

autologous use in the treatment of cerebral palsy and the regressive form of autism ([105] and H Brown pers. commun.). The University of Texas at Houston has begun an FDA-approved clinical trial to treat children with traumatic brain injury using autologous bone marrow stem cell infusions (Cox *et al.* [106], UT Health Sciences Center, Houston, Safety of Autologous Stem Cell Treatment for Traumatic Brain Injury in Children). Although this latter study does not use CB stem cells, comparable cell populations are found in CB and could be similarly applied. Further, it has been recently suggested that CB stem cells will be used for the clinical treatment of stroke as well as the treatment of spinal cord injured patients (a 300-patient trial for spinal cord injury is to begin in China in 2007 according to AABB SmartBrief updates).

4.4 Orthopedic: cartilage and bone

Although many researchers have investigated the use of bone marrow MSC for the repair of bone and cartilage, analysis of the potential of CB stem cells has been under examined. As orthopedic applications could present a tremendous opportunity for CB stem cells to rapidly migrate to the clinic, additional studies should be expected in the near future. It is estimated that > 1 million individuals in the US annually suffer from articular joint injuries involving cartilage, ligaments and/or tendons, as well as difficult to heal bone fractures [107]. As CB contains both ES-like stem cells as well as MSC one should not be surprised that it is capable of differentiating into both bone and cartilage. In fact, in animal models MSC from bone marrow has been shown to give rise to these tissues *in vivo* [59]. CB stem cells have also been shown to be capable of becoming bone *in vitro* when subjected to shockwave induction [60]. In fact, when these cells were placed into animals with fractured femurs there was significant bone healing. Along these lines autologous bone marrow MSC have been used to treat torn patellar cartilage in patients with resultant long-term functional improvements [61]. Work from the laboratories of Szivek and Harris have also examined the ability of CB stem cells to become cartilage in comparison to tissues derived from bone marrow MSC and adipose stem cells, with early encouraging results (J Szivek pers. commun. and [62•]). Finally, several laboratories are working on using both bone marrow and CB stem cells for ligament repair (200,000 cases annually in the US). In fact, a cottage industry has started up to bank CB stem cells as a sports injury repair kit for the future [108]. Similarly, several companies have commercialized this approach in the veterinary field, offering this service for thoroughbred horses, with significant clinical success [63•].

4.5 Eye disease

Regenerative medicine for the eye involves reconstruction of the cornea or the retina. The cornea appears to be the most suitable for routine clinical applications, and may involve either the epithelial and/or the endothelial cell layers. The outer layer of the eye is made up of the central cornea, the limbus and the sclera. The cornea epithelium is a rapidly self-renewing tissue; implicated to have its own source of stem cells (the limbus) specialized for this purpose. Corneal epithelial stem cell deficiency is an important cause of visual disability. Many disorders lead to the loss of corneal epithelial stem cells such as alkali injury, Stevens–Johnson syndrome, ocular cicatricial pemphegoid, aniridia, chronic rosacea keratoconjunctivitis and iatrogenic causes. Without a normal corneal epithelium, a clear image cannot be focused on the retina. Autologous corneal epithelial stem cell grafts have been successful for patients with unilateral disease. However, harvesting cells from the functional eye places the healthy eye at risk for vision loss. Additionally, in bilateral conditions, autologous grafts are not available. The best solution at present for bilateral disease is a corneal epithelial stem cell allograft. Allografts require chronic antirejection therapy with possible systemic side effects. In addition, the average survival of allografted corneal stem cells is two years. Lack of adequate tissue for patients with corneal surface disease is a problem because until such tissue is identified, visual rehabilitation will be impossible. Severe corneal wounds requiring intervention are not uncommon. In fact, corneal wounds make up 37% of all visual disabilities and almost a quarter of all medical visits for ocular problems in North America [64,65•].

Although not a limiting factor in the US, a scarcity of cornea donors is often a problem in Asian countries. In fact, in Japan alone it is estimated that there are 20,000 – 50,000 patients annually awaiting cornea transplant with only 1500 donors available. Work from the group of Nichols *et al.* [66-68] have used CB stem cells as a viable therapeutic modality for ocular surface disease, as differentiated human CB stem cells represent an unlimited source of tissue for ocular surface reconstruction. Although there is no extensive published literature regarding the use of CB stem cells for ocular surface reconstruction, preliminary laboratory and animal data are supportive of this hypothesis. Histology and immunohistochemistry of the differentiated CB stem cells revealed that the resultant cell sheet was morphologically indistinguishable from corneal epithelial cells. CB stem cells were capable of expressing the corneal epithelial specific cytokeratin, k3. When New Zealand white rabbits were transplanted with the cell sheets it was able to reconstitute the cornea, forming an optically clear surface (see **Figure 2**). Significantly, this work implicated the existence of multiple progenitor cell populations in CB capable of becoming corneal epithelium.

Other investigators have demonstrated that MSC are also capable of reconstituting the cornea in a rat model [69]. In a rat model of chemically induced loss of the cornea, human bone marrow-derived MSC could replace it and appeared to work similar to limbal stem cells. As CB also contains MSC, this observation may partially explain the mechanism of action of CB stem cells in the previous application.

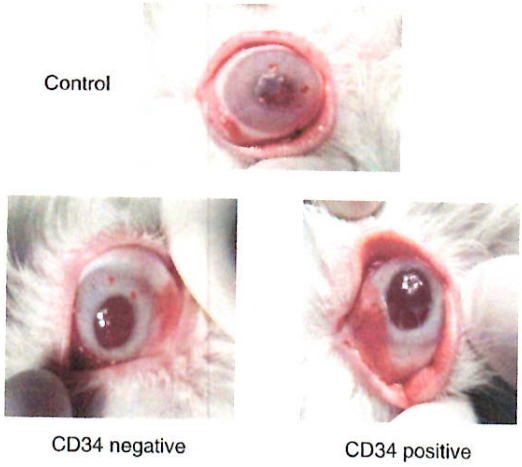

Figure 2. Demonstration of multiple epithelial cell progenitor populations in CB by *in vivo* transplantation of CB-derived corneal tissue. CB stem cells (CD34-enriched or CD34-depleted) were cultured *in vitro* on amniotic membranes with growth factors and then transplanted onto denuded rabbit eyes. Tissues were examined 2 weeks post-transplant.
CB: Cord blood.

Table 2. Comparison of stem cell characteristics.

	CBSC	Adult	ES cells
Capacity to differentiate into various cell types	✓	✓	✓
Highly proliferative	✓		✓
Very low risk of viral contamination	✓		
Immediately available	✓	✓	
Allow for autologous transplants	✓	✓	
Established/proven treatment in human patients	✓	✓	

CBSC: Cord blood stem cells, ES: Embryonic stem.

4.6 Other applications

4.6.1 *Liver damage*

The liver is the one organ in the human body capable of significantly regenerating itself. Thus, it may provide the ideal niche for exogenous stem cells to differentiate into hepatic tissue for regenerative medicine applications. In fact, Kakinuma *et al.* [70] have shown in mice that animals which were subjected to partial hepatecomy and received a human CB transplant exhibited engrafted cells in the liver: these cells became hepatocytes, and were capable of secreting albumin for up to one year postinfusion. Similar results were obtained in a chemically induced animal model of liver disease in which infused CB stem cells once again differentiated into mature hepatocytes and helped to regenerate the liver, as compared with control animals [71]. Recent work has implicated MSC present in the CB population as being at least partially responsible for this effect [72].

4.6.2 *Lung*

Recently, it has been demonstrated that CB progenitor cells could be driven to become respiratory epithelial cells [73]. This observation might not be too surprising in that CB stem cells are known to also be able to become corneal epithelial tissue. In culture the CB stem cells became type II alveolar cells which made surfactant protein C (specific for type II lung cells). Thus, CB stem cells might be amenable to regenerative medicine for various lung diseases (e.g., emphysema or cystic fibrosis).

4.6.3 *Wound repair*

As CB stem cells could become both corneal and respiratory epithelial cells, it might not be unexpected that these cells could also differentiate into skin epithelial cells and thus be useful in wound repair (e.g., diabetic ulcers). Work from the Harris and Ablin laboratories has begun to investigate this premise, knowing that previous studies have demonstrated a bone marrow stem cell contribution to wound healing in mice (presumably due to MSC, R Ablin pers. commun.) [74]. In agreement with this hypothesis, in 2004 there was an initial report of the use of allogeneic CB $CD34^+$ progenitor cells in two patients to promote skin wound/lesion repair [75]. In this instance the progenitor cells were admixed with an autologous fibrin matrix and 3×10^4 cells were injected in a volume of 3 ml into the margins of the lesions. At 3 – 7 months follow-up there was no sign of graft versus host disease and, most importantly, there were evidence of significant healing in both patients.

5. Expert opinion and conclusion

Regenerative medicine has the ability to prevent many of the above discussed conditions by replacing or repairing malfunctioning tissues. At present, US healthcare costs are > $1.5 trillion annually, or 13% of GDP. A large fraction of these costs is attributable to tissue loss or organ failure, with ~ 8 million surgical procedures being performed annually in the US to treat these disorders. By the year 2040, the population of senior citizens in the US will be double today's number, for a total of 70 million. As much as 25% of the US GDP could be devoted to healthcare by the year 2040. Because regenerative medicine focuses on functional restoration of damaged tissues, not necessarily abatement or moderation of symptoms, this field cuts healthcare costs. Without regenerative medicine, the US faces a future of rising healthcare costs and inefficient medical treatments. In the US 250,000 patients receive heart valves annually, at a cost of US$27 billion. Another 950,000 people die of heart disease or stroke, at a cost of $351 billion annually. Finally, 17 million patients live with diabetes and its complications, at a cost of $132 billion annually.

One should be cognizant of the fact that ES cells, regardless of your ethical, moral or political bent, have numerous biologic roadblocks/limitations that will prevent their trek to the clinic and access to patients for some years. These limitations include inherent allogenicity and the threat of immune rejection; thereby creating the need for therapeutic cloning (which may not be applicable to long-lived species) to create patient-specific ES cells and ES therapies; and due to the relatively low efficiency of cloning the need for extremely large numbers of egg donors if and when therapeutic cloning is realized. In addition, the threat of teratoma formation if ES cells are directly used in patients without first differentiating the ES cells to the desired tissue; the inability to derive these differentiated cell lines in time to meet the treatment windows for successful therapies to be realized as most patients will present with a limited time to treat; and finally, the overall costs of overcoming each of these limitations may ultimately limit both the progress of ES cell therapies to the clinic and access to the population in general.

Non-ES cells, however, can generally be obtained from the patient themselves (autologous) and readily used in an expedient and economically efficient manner. Regenerative medicine requires immediate access to easily obtainable and large numbers of pluripotent stem cells. Such non-ES cell sources that are readily obtainable in large numbers for most individuals include the bone marrow, adipose tissue and umbilical CB. Of these three sources, we believe that CB stem cells are the best alternative to ES cells in that they appear capable of being used in many of the same applications claimed for ES cells, including cardiac, neurologic, orthopedic and ophthalmic applications (see **Table 2**).

Cumulatively, the ever-expanding body of CB stem cell research shows that CB has moved past its traditional hematologic applications into the field of regenerative medicine. This evolution in CB use has been demonstrated by the discovery of CB pluripotency, and the identification of primitive, plastic stem cells within CB. However, the true test of therapeutic potential is the ability of these cells to engraft in a living organism and produce measurable improvement in disease states. CB has shown this ability in therapeutic areas that impact nearly every American, including heart disease, neurologic damage, diabetes, vision loss and liver damage; in addition to its use to treat thousands of patients annually with blood disorders and malignancies.

Already, in the cases of Type I diabetes and neurologic (cerebral palsy and anoxic brain injury) applications, CB research has transitioned from the laboratory to the clinic, and patients are now being treated in clinical trials. Clinical trials are beginning for patients with peripheral vascular disease, non-healing wounds and spinal cord injury. Other trials will surely follow in a timely manner, including therapies for stroke, the eye, liver and joints. Further into the future we would expect to see treatments for myocardial infarction and other forms of cardiovascular disease, as well as Parkinson's disease. However, the key to these advances lies in the ability of CB stem cells to be used in many instances under the practice of medicine (rather than under an investigational new drug) as it appears in many systems that it is possible to merely infuse the stem cells directly without timely and costly *in vitro* culture and differentiation steps. This assertion is supported by the clinical trials for Type I diabetes and cerebral palsy discussed above.

To take advantage of these applications and opportunities individuals must elect to store autologous CB collections, which involves some costs (see [105] for more details). However, these upfront costs are comparable to the costs of adipose stem cells harvests, are less than the costs of bone marrow collections or ES cell derivations, and when amortized over the lifetime of the individual, are miniscule. The samples, once cryopreserved, are capable of being stored indefinitely and therefore used at any point during the individual's lifetime. Although the numbers of stem cells obtained from a typical CB collection are limited and available only once, recent developments in stem cell expansion should obviate this concern in short order. Thus, very soon it will be possible to obtain multiple regenerative uses from a single CB collection. In this regard, it should be noted that for many of the cerebral palsy applications only a small fraction of the CB harvests were needed for therapy (see [105] for additional details).

The magnitude and promise of these findings will continue to transform medical thought for the foreseeable future as these stem cells become more and more recognized at present as the best alternative to ES cells. We believe that research and clinical trials conducted over the next several years will demonstrate that CB stem cells are capable of performing most if not all of the function of ES cells, and that CB stem cells may eventually render the current ES cell debate mute. But most important of all, CB stem cells will undoubtedly treat thousands of patients via regenerative medicine before a single patient is treated in an ES cell therapy.

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