

Chapter 2

Regenerative Potential of Cord Blood

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1 Introduction

The field of regenerative medicine is dedicated to the study of repairing, replacing, or regenerating damaged human cells, tissues, or organs to restore or establish normal function [1]. This could be approached through numerous strategies, from stimulating endogenous processes to repairing damaged tissue to deriving or transplanting entire organs to replace those that are beyond repair. Though the field is currently in its infancy, regenerative medicine is predicted to be one of the most important disciplines in the next decade, with applications in a wide variety of conditions. Potential cells that could serve as source materials for regenerative medicine and cellular therapies include hematopoietic stem and progenitor cells derived from bone marrow (BM) or umbilical cord blood (CB), placental and amniotic fluid and tissues, mesenchymal stromal cells (MSCs), skin cells, and other organ-specific cells that could be engineered to perform reparative functions. This chapter will explore some of the potential regenerative applications for which CB could serve as a valuable source of cells.

2 Umbilical Cord Blood (UCB) as a Source of Stem Cells for Regenerative Applications

Human CB is rich in highly proliferative stem and progenitor cells mobilized by placental signals promoting homing to developing organs [2, 3]. CB is readily available, can be collected noninvasively without risk to the mother or infant donor, and can be cryopreserved for several decades for future use. Compared to stem cells obtained from adult BM, CB stem cells are less mature and therefore have longer telomeres and greater proliferating potential [4]. They are also less immunogenic and less likely to transmit infections via latent viruses. In more than 25 years of use in allogeneic,

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unrelated hematopoietic stem cell transplant (HSCT), CB has not been shown to cause any teratomas or solid tumors. CB is often discarded as medical waste with the placenta after birth. Recently, induced pluripotent stem (iPS) cells have been isolated from CB with simpler methods and greater efficiency as compared to adult cell sources [5–7].

CB is a well-established source of stem cells for hematopoietic rescue after myeloablative HSCT. In addition, CB also contains nonhematopoietic stem cell populations with the ability to differentiate into numerous cell types throughout the body. In particular, the CB-derived unrestricted somatic stem cell (USSC) first described by Kogler et al. is a nonhematopoietic multipotent cell with the ability to differentiate into several lineages in vitro and in vivo [8]. USSCs can give rise to cell types from all three germinal layers, including osteoclasts [8], hepatocytes [9], and neurons [10], among others. CB-derived stem cells can also differentiate into MSCs [11], chondrocytes [12–14], osteocytes [13–18], adipocytes [14–17], neural cells [14, 15, 18–20], myocytes [21], hepatocytes [14, 15], pancreatic cells [22], cardiomyocytes, skin cells, and endothelial colony forming cells (ECFCs).

CB donor-derived tissue-specific cells have been identified in multiple organs in both animals and humans after HSCT, including the liver [19], lung, pancreas [19, 23], skeletal muscle [24], and brain [13]. In addition, transplanted lineage-negative human CB cells with high aldehyde dehydrogenase activity ($\text{ALDH}^{\text{hi}}\text{Lin}^{-}$) have been detected in several nonhematopoietic tissues in mice, including the liver, lung, kidney, heart, pancreas, cartilage, brain, and retina [19]. While CB cells have the ability to differentiate into tissue-specific cells and integrate into host organs, there is growing evidence that their therapeutic effects may stem more from their ability to initiate tissue repair by activating host cells via paracrine effects. Nonetheless, these observations indicate that transplanted CB cells are capable of repopulating more than just the hematopoietic system [25, 26]. This may be due to the presence of a true embryonic-like stem cell in CB or small numbers of committed tissue-specific, nonhematopoietic progenitors.

The pluripotential nature of CB, as well as the relative ease of collection, processing, testing, and storage, make it an attractive source of cells for regenerative medicine applications across many disciplines, including neurology, cardiology, orthopedics, endocrinology, and others. In this chapter, numerous preclinical, animal, and human studies evaluating the use of CB and CB-derived products across a wide range of clinical conditions will be reviewed.

3 Cord Blood (CB) Therapies in Neurological Diseases

Neurologic impairment can result from acquired injuries, genetic conditions, or neurodegenerative diseases of unclear etiology. Recovery from neurological injuries is typically incomplete and often results in significant and permanent disabilities. Currently, most available therapies are limited to supportive or palliative measures, aimed at managing the symptoms of the condition. Since restorative therapies targeting the underlying cause of most neurological diseases do not exist, cell therapies

targeting anti-inflammatory, neuroprotective, and regenerative potential hold great promise. CB cells can induce repair through mechanisms that involve trophic or cell-based paracrine effects or cellular integration and differentiation. Both may be operative in CB therapies for neurologic conditions, and there are numerous potential applications of CB-based regenerative therapies in neurological diseases, including genetic diseases of childhood, ischemic events such as stroke, and neurodegenerative diseases of adulthood.

Multiple *in vitro* studies have demonstrated that neurons, astrocytes, oligodendrocytes, and microglia can be derived from CB cells via gene transfection, *ex vivo* culture with growth factor supplementation, and/or the use of chemical agents [20, 27–34]. Neural differentiation has been documented in phenotypic and functional assays. The phenotype of the derived cells has been characterized by gene arrays [35] and the expression of standard neural-specific markers and proteins. Additionally, functional characteristics have been demonstrated through the presence of voltage- and ligand-gated ion channels with the ability to conduct electrical activity, indicating the development of functional characteristics of neurons [31].

The mechanisms of repair are expected to vary between indications, and several possibilities have been hypothesized [36]. Transplanted cells may deliver trophic factors that provide anti-inflammatory and neuroprotective effects and enhance the survival potential of host cells [37–40]. They may increase the plasticity of the injured brain by enhancing synaptogenesis, angiogenesis resulting in neovascularization, endogenous repair mechanisms, and migration and proliferation of endogenous neural stem cells [41–43]. Stem cells may also migrate, proliferate, and differentiate into “replacement” neuronal and glial cells and play a role in remyelination [44]. Additionally, many neurologic diseases involve activation of proapoptotic signal transduction, which could be harnessed to attract cells to brain lesions in those diseases. Thus, CB-derived cells could also potentially act as a vehicle to deliver neuroprotective and restorative factors in a targeted way toward damaged brain tissue.

3.1 Genetic Brain Diseases in Children

As discussed in greater detail in the chapter by Dr. Prasad, allogeneic transplantation of human CB in patients with certain genetic lysosomal and peroxisomal storage diseases is effective in preventing or ameliorating the associated neurological damage [45–48]. The engraftment of donor cells into a patient with an inherited metabolic disease provides a constant source of enzyme replacement, thereby slowing or halting the progression of disease. Patients with these diseases, ranging in age from newborns to young adults, transplanted early in the course of their disease derive extensive benefits from the transplant procedure, which both extends life for decades and greatly improves neurologic functioning [49–51]. Clinical and pathological observations from these patients provide additional support for the concept that CB cells can repair nonhematopoietic tissues.

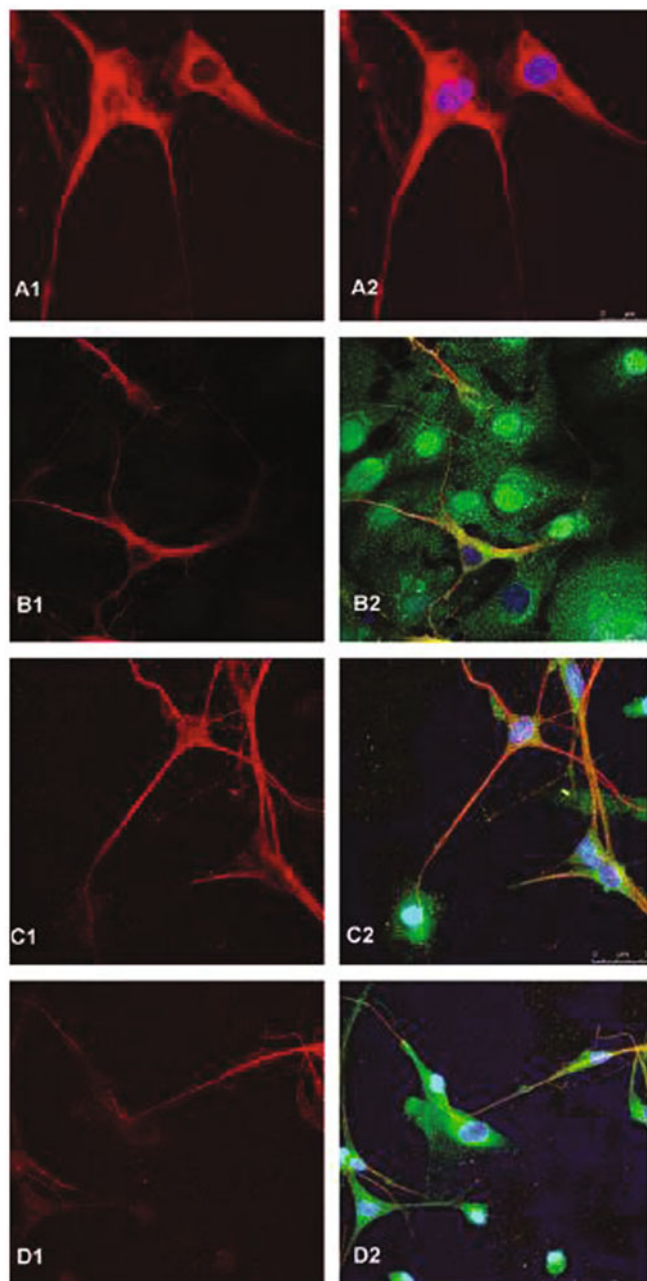


Fig. 2.1 In vitro functional assay of myelination of shiverer mouse neurons by cryopreserved cord-blood-derived oligodendrocyte-like cells (*O-cells*). Shiverer neurons cocultured with *O-cells* were co-stained for BT3 (Texas Red) and MBP (fluorescein isothiocyanate). Controls stained positive for BT3 (panel A1) but not MBP (A2). When cocultured with *O-cells* for 1 week, BT3 (B1) and MBP (B2) were expressed. Z-stacked projection after 3 weeks in culture demonstrated BT3 expression (C1, D1) and close association between BT3-expressing neuronal cells and MBP-expressing cells (C2), with MBP expression along axonal processes (D2)

Autopsy studies in humans who died after intravenously administered, sex-mismatched BM and CB transplant have confirmed the engraftment of donor cells throughout the brain months after transplantation [52–54]. Most engrafting cells were nonneuronal microglial cells, but donor-derived neurons, astrocytes, and oligodendrocytes have been identified. Globoid bodies, the pathological perivascular signature of Krabbe disease, were not detected in a patient transplanted for early infantile Krabbe disease at 3 weeks of age who died of unrelated causes at 5 years of age [54]. Based on these observations, our group hypothesized that CB contained cells capable of differentiating into oligodendrocyte-like (“O-cells”) and microglial-like cells. We subsequently cultured and expanded O-cells from fresh and cryopreserved CB after 3–4 weeks in tissue culture supplemented with neurotrophic growth factors [20, 28]. These cells grow as an adherent population that, after 21 days in culture, express surface antigens found on oligodendrocytes (O1, O4, Proteolipid Protein (PLP), Myelin Basic Protein (MBP)) and microglia (CD45, CD116), make corresponding RNAs, and myelinate shiverer neuron axons in an in vitro potency assay (Fig. 2.1). They also constitutively produce IL-6 and IL-10 and retain the ability to produce lysosomal enzymes in culture after manufacturing. Intrathecal dosing in immunodeficient newborn mice showed the best distribution of O-cells in the central nervous system. A phase I trial administering these cells intrathecally 1 month after a standard HSCT from the same CB donor is planned. This trial is one example that the availability of well-characterized, screened, and HLA-typed CB coupled with its vast differentiation potential makes it an attractive source of stem cells for applications in tissue repair and regeneration, particularly in the central nervous system.

3.2 Ischemic Injuries

Observations using CB to treat children with genetic conditions led to the hypothesis that CB might also be beneficial in patients with brain injury. Accordingly, CB cells have been investigated in preclinical models of stroke, neonatal hypoxic-ischemic encephalopathy (HIE), traumatic brain injury, and spinal cord injury. These injuries are typically characterized by immediate damage to all neural cell types within the affected region. Therefore, therapeutic strategies might involve methods to promote cell survival and repair or regeneration of the affected areas, potentially via anti-inflammatory effects, neurogenesis, synaptogenesis, and/or angiogenesis after the injury has been sustained.

Numerous animal models have demonstrated both neurological and survival benefits of CB cells in the setting of stroke, ischemia, intracranial hemorrhage, and spinal cord injury [55–61]. Neuroprotection [55], neovascularization [43], and neuronal regeneration [62] have all been demonstrated in various models. For example, in HIE, a neonatal rat model has been developed by unilateral carotid artery ligation on day seven of life. Without intervention, these animals universally develop severe cerebral damage and contralateral spastic paresis. Meier and Jensen administered human CB mononuclear cells to these animals intraperitoneally 1 day after the hypoxic event, showing that the cells migrate to the area of brain damage and persist

for at least 2 weeks. Although the extent of morphologic injury on gross pathology was not altered, animals who received CB mononuclear cells did not develop spastic paresis, indicating functional recovery [57]. In a baby rabbit model of HIE [63], Tan demonstrated that labeled human CB cells reached the brain within 24 h, persisted for at least a week, and decreased the degree of brain damage on magnetic resonance imaging (MRI). In severely affected animals, CB administration improved gross motor function in a short-term functional assay [64]. Additionally, Ballabh and colleagues developed a rabbit model of intraventricular hemorrhage (IVH) by administering glycerol intraperitoneally to premature rabbit pups [65]. In this model, IVH is followed by the development of hydrocephalus and subsequent white matter demyelination. Intraventricular administration of human CB cells 24 and 72 h after glycerol failed to prevent the hydrocephalus, but did reduce subsequent demyelination (Ballabh, personal communication, 2014).

The therapeutic potential of intravenous infusions of autologous CB is currently being investigated in young children with cerebral palsy, HIE, and traumatic brain injury. In a safety study, we treated 184 infants and children with cerebral palsy (76 %), congenital hydrocephalus (12 %), and other brain injuries (12 %) with intravenous autologous CB infusions [66]. Patients were treated in the outpatient clinic through a peripheral IV after a single dose of Tylenol, Benadryl, and Solumedrol. Approximately 1.5 % of patients experienced hypersensitivity reactions (i.e., hives and/or wheezing) during the CB infusion that resolved after discontinuation of the infusion and outpatient medical management. With more than 3 years of follow-up, no additional adverse events have been reported, indicating that the procedure is safe. Parental reports of improved function were common, but it was difficult to know whether these improvements were directly related to the infusion of CB cells. Thus, a randomized, double blind, placebo-controlled study is in process to determine the efficacy of this approach. In this study, children ages 1–6 years are randomly assigned to the order in which they receive CB and placebo infusions, each given 1 year apart (Fig. 2.2). Motor, cognitive, and imaging studies are performed at baseline and 1 and 2 years to evaluate any differences between CB and placebo groups. The primary endpoint is improvement in motor function on standardized scales. A similar study of allogeneic CB and erythropoietin was conducted in Korean children with cerebral palsy [67]. They reported greater improvements in cognitive and select motor functions in children who received CB and erythropoietin versus controls. There was no CB-only group for comparison.

In the USA, CB is being investigated in clinical trials for children with cerebral palsy, neonatal hypoxic ischemic encephalopathy, stroke, traumatic brain injury, and autism (NCT01072370, NCT01700166, NCT01988584, NCT01251003, NCT01638819). Studies administering CB cells intravenously or intrathecally are being conducted in children with brain injury in other countries as well. Of note, intrathecal administration of allogeneic CB-derived cells, mostly MSCs, has been performed for a variety of neurologic conditions in a few small studies, primarily in China. In general, side effects are reported to be minor and transient, most commonly including fever, headache, and dizziness [68–71]. Efficacy cannot be determined at this point.

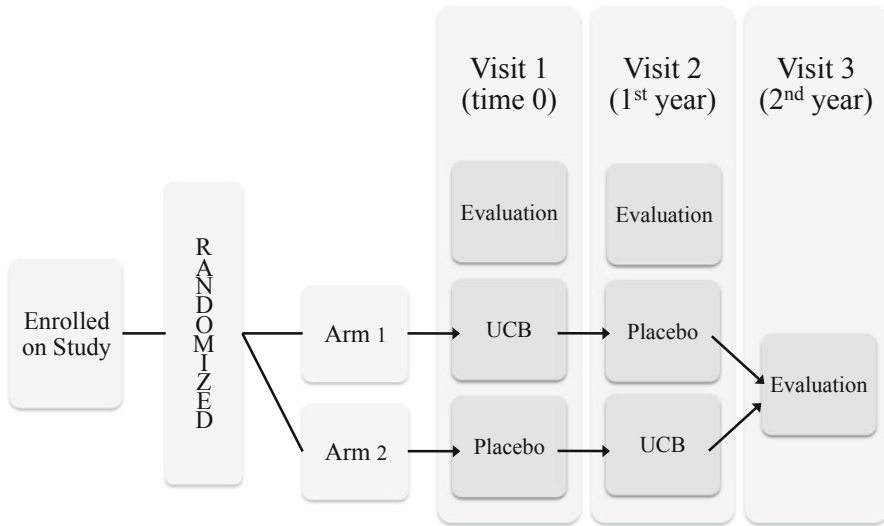


Fig. 2.2 Study design of a randomized, double blind, placebo blind, placebo-controlled trial of an intravenous infusion of autologous CB in children with cerebral palsy. Endpoints include changes in functional status (gross motor, fine motor, speech, cognition), quality of life, and neuroimaging (brain MRI with DTI, fibertracking, and connectivity analyses [139])

In phase I trial of newborns with hypoxic ischemic brain injury at birth conducted at Duke University, fresh, noncryopreserved autologous CB processed on Sepax 1 (Biosafe, Geneva) was infused in 1, 2, or 4 doses within the first 72 h of life in babies with moderate-to-severe encephalopathy qualifying for systemic hypothermia [72]. These babies were compared to a concomitant group of babies who were cooled at Duke but did not receive CB cells. Infusions were found to be safe in these critically ill babies, and babies receiving cells had increased survival rates to discharge (100 % vs. 85 %, $p = 0.20$) and improved function at 1 year of age (74 % vs. 41 % with development in the normal range, $p = 0.05$). A phase II randomized trial is currently in development. If this therapy improves the outcome of babies with significant birth trauma, there could be potential implications for the current model of CB collection and banking. In order to make this therapy available to all eligible babies, at a minimum all obstetric providers would need to be trained in CB collection, CB would need to be routinely collected at at-risk deliveries, and centers would need to either process and infuse the CB or transfer the babies and their CB to centers that have the ability to do so. Alternatively, CB collection could become a routine practice at every delivery. Units could then be stored for a limited time until it is clear if the baby will need it. If not, the autologous CB units could then be discarded, used for research or other regenerative applications, or, if appropriate consent and testing were obtained, transferred to the public registry.

Most human studies of stem cells in adults who have suffered a stroke have utilized autologous BM cells [73, 74]. Though no safety concerns have been identified, the studies are too small to reliably assess efficacy, and investigations of clinical

endpoints are currently underway. However, as the majority of adult stroke victims are elderly and critically ill following their injury, a CB-derived off-the-shelf therapy is an attractive alternative to autologous BM as it would avoid the need for a potentially risky BM harvest in these critically ill patients. In addition, these frequently elderly patients may also have decreased progenitor cells and other conditions that may limit the functionality of their own BM cells [75, 76]. A few clinical trials of CB cells given intravenously or intraparenchymally into the brain (NCT01700166, USA; NCT01884155, Korea; NCT01673932, China) are accruing patients at the present time but no results have been published to date.

3.3 Neurodegenerative Diseases

Cellular approaches to neurodegenerative diseases have been studied most extensively in Parkinson's disease, in which degeneration of primarily nigrostriatal dopaminergic neurons results in motor symptoms such as resting tremors, rigidity, and hypokinesia. Cell-replacement strategies have been attempted in over 300 patients with Parkinson's disease using intrastriatal implantation of fetal mesencephalic tissue. While several open-label trials suggested clinical benefit, two double-blind studies did not find a significant effect [77, 78]. However, some patients have demonstrated durable improvements including the ability to withdraw dopaminergic medication, and clinical benefit has been seen preferentially in less-disabled patients. Given the limitations of using human fetal tissue, interest has grown in generating dopaminergic neurons from other cell sources. Neurons that express dopamine-related genes and demonstrate the ability to synthesize and release dopamine have been successfully derived from CB stem cells in vitro [29, 30]. In hemiparkinsonian rats, unmodified human umbilical cord MSCs injected into the striatum can improve behavioral symptoms, and this effect is enhanced by adenovirus-mediated VEGF modification of the cells [79]. These studies indicate that CB has potential as a source of stem cells for cellular replacement strategies in Parkinson's disease.

CB cells have been evaluated in in vitro and in vivo models of Alzheimer's disease. Transgenic mice treated with CB-MSCs show a reduction in both microglial activation and beta-amyloid deposits, the pathologic signature of the disease [80]. CB-treated mice also demonstrate decreased cognitive impairment in functional assays [81] and an extended lifespan [82]. Although the mechanism is not entirely clear, it is possible that the CB cells mediate the microglial response to beta-amyloid deposits, promote beta-amyloid phagocytosis, and/or prevent apoptosis of host cells. Neurostem®, a CB-MSc product, has been investigated in a phase I trial in Korea, though results of that study have not yet been published.

3.4 Autism

Emerging data suggest that autism is caused by a complex interaction of genetic and environmental conditions, resulting in abnormal brain functioning early in life.

Recently, stem cell therapy has become appealing as a potential therapy to many within the autism community. In a mouse model of autism, intraventricular administration of human adipose-derived stem cells resulted in decreased repetitive movements and improved social activity [83]. Clinical trials of autologous BM and CB in children with autism are being conducted in the USA (NCT01638819), Mexico (NCT01740869), China (NCT01343511) [84], and India (NCT01974973, NCT01836562) [85]. Due to the heterogeneity in both etiology and symptomology of autism, identifying appropriate subjects and outcome measures remain particularly challenging.

4 Cord Blood (CB) Therapies in Cardiovascular Diseases

Regenerative medicine may have a role in multiple types of cardiac disease, including ischemic damage, heart failure, and even engineering replacement heart valves [86]. Research, primarily utilizing human BM-derived cells, has thus far focused on attempting to minimize the effects of myocardial infarction (MI), most commonly via intracoronary injection of stem cells. Numerous animal models have shown that BM-MSCs and/or MSCs can improve myocardial perfusion, reduce infarction scar size, and decrease left ventricular remodeling [87–89]. In human studies, over 2600 patients with acute or chronic ischemic heart disease have been safely treated with autologous BM cells. A meta-analysis of 50 such studies concluded that BM-MSCs not only improve left ventricular function, infarct size, and remodeling in patients with ischemic heart disease, but also result in an increased rate of survival and decreased rates of recurrent MI and stent thrombosis [90]. For acute MIs, the therapy seems to be more efficacious when given early (i.e., within 7 days of MI), though benefits were still seen when cells were administered 7–30 days after the ischemic event. A dose-effect was also demonstrated, with $< 40 \times 10^6$ cells resulting in no improvement. A phase III randomized controlled study of autologous BM-MSCs after acute MI is planned (NCT01569178). An allogeneic BM-MSC product (Prochymal; Osiris) was also shown to be safe when delivered intravenously after acute MI, and is currently under investigation in a phase II study (NCT00877903).

CB stem cells could offer advantages over patient-derived stem cells, including longer lifespan and proliferation potential and avoidance of cytokine mobilization and harvesting procedures in patients who are inherently at increased cardiovascular risk. CB-derived cells have been successfully differentiated into cardiomyocytes in vitro [91], and human CB-derived cardiomyocytes have been demonstrated in the ventricles, septum, and Purkinje fiber system of preimmune fetal sheep after intrauterine transplantation [8] and in the myocardium of rats after intracoronary injection [92]. However, direct replacement of damaged myocardium by the differentiation and engraftment of CB or other hematopoietic cells is unlikely to be the main mechanism by which stem cells can aid in repair. In rodent and porcine models of acute and chronic MI, transplanted CB cells demonstrate improvement in cardiac function despite surviving at most 2 months [93–96]. This suggests that CB cells

can modulate the myocardial response to injury, thereby inducing preservation or regeneration of host myocardium and ultimately resulting in decreased scar formation and improved functional recovery. Potential mechanisms of action include the release of cytokines and growth factors that promote cytoprotection and neovascularization, activation of host cardiac stem cells to regenerate, and/or recruitment of stem cells from other tissues (i.e., BM) to differentiate into replacement cardiomyocytes. CB-MSCs are being investigated in clinical trials for dilated cardiomyopathy (NCT01739777, Chile) and ischemic disease (NCT01946048, China).

Regardless of the cell source, there are site-specific challenges for cellular regenerative techniques in cardiovascular diseases. The heart is subject to high blood flow velocity, so the majority of stem cells administered intravascularly can easily be swept away in the circulation before they are able to take hold in the desired location. Additionally, therapeutic cells may have decreased survival when delivered to an ischemic environment. Techniques to enhance cell homing, recruitment, and retention in the damaged area may improve the effectiveness of the delivered cells.

Stem cell therapies are also under investigation for critical limb ischemia, which occurs as a result of severe peripheral vascular disease. Asahara first demonstrated the existence of circulating hematopoietic lineage endothelial precursors (EPCs) that express the surface markers CD34, CD133, and Flk-1/KDR, can differentiate into mature endothelial cells *in vitro*, and contribute to vessel formation after transplantation [97]. Given their close relationship and numerous shared surface markers with hematopoietic stem cells, it is difficult to distinguish the two cell populations. In preclinical models, BM or CB cell administration has resulted in improved vascularization and symptoms [98–101]. Human studies have been conducted with BM-derived cells, showing improvement in symptoms and capillary density [102–105]. Intramuscular CB cell administration for limb ischemia has been reported in a few small case reports [106, 107] in which the therapy was well tolerated and some patients demonstrated improvement such as healing ulcers. However, the samples are too small to draw conclusions regarding efficacy, and a larger study is underway (NCT01019681, USA). Methods of administration have included intramuscular and intra-arterial injections, and the cell dose, number of doses, and reported duration of effect vary.

5 Cord Blood (CB) Therapies in Bone and Collagen Diseases

Musculoskeletal problems including bone defects and nonunions, avascular necrosis, and arthritides are common, and they continue to increase along with the elderly population, the obesity epidemic, and orthopedic advances that allow for limb-sparing procedures. Autologous bone grafting is a standard treatment approach in multiple scenarios, but it requires harvesting bone from another location (often the posterior iliac crest) and suffers from decreased regenerative potential of the graft in elderly patients. Several bone substitutes, synthetic or natural, have been utilized, but they typically decrease the rate of osteogenesis and carry all the risks of implanted foreign bodies. Therefore, cellular therapies have elicited great interest for use in bone and cartilage regeneration.

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