

PERINATAL MESENCHYMAL STEM CELL BANKING FOR UMBILICAL CORD BLOOD TRANSPLANTATION AND REGENERATIVE MEDICINE

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INTRODUCTION

Traditionally, bone marrow (BM) and mobilized peripheral blood (MPB) have been the predominant source of autologous and allogeneic hematopoietic stem cells (HSCs) for transplantation of patients diagnosed with BM and blood disorders. Over the past several decades, however, umbilical cord blood (UCB) has been established as an alternative source to BM and MPB for HSC transplantations. HSCs found in the human UCB have demonstrated clinical benefit, as exemplified by their use in over 20,000 transplantations worldwide in humans for the treatment of a host of hematopoietic and nonhematopoietic diseases. As a result of their clinical potential, over the past 20 years, UCB from newborns have been cryopreserved and banked, both publicly and privately, for clinical use. However, the transplantation of UCB cells is limited by the finite number of derivable HSCs from each unit. Strategies to overcome this shortcoming have included *ex vivo* expansion of HSCs, as well as, multiple UCB unit transplantations, both with limited success. Specifically, there has been no clinical evidence to date for the successful *ex vivo* expansion of HSCs, and multiple UCB unit transplantations are limited by unit quality, availability, and the appropriate HLA match. A more realistic path to overcome the clinical limitations of UCB is the cotransplantation of mesenchymal stem cells (MSCs), specifically those derived from the umbilical cord (UC) tissue, along with UCB. Recent studies have shown an increase in hematopoietic engraftment when UC-derived MSCs are coinfused with UCB, compared with UCB infusions alone. As a result, technologies to process and cryopreserve MSCs found within the UC tissue have been established. Thus, both MSCs and

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HSCs from the UC from the same newborn are currently banked for future use in treating various disease indications, in both hematopoietic and nonhematopoietic regenerative medicine applications.

HEMATOPOIESIS

Hematopoiesis in the adult human takes place in the BM cavity, constantly being rejuvenated by two main populations of stem cells that have specific, yet different, roles in regulating hematopoietic homeostasis. The HSC possesses the long-term memory for the production and differentiation of both myeloid (e.g., erythrocytes, megakaryocytes, and granulocytes) and lymphoid (e.g., T & B lymphocytes) cell types. MSCs—also found within the bone marrow cavity—possess the long-term memory for the production of mesodermal cell types (e.g., osteocytes, adipocytes, chondrocytes, and myocytes). Additionally, MSCs provide support for HSCs—and hematopoiesis—since they produce essential paracrine factors (cytokines, growth factors, chemokines, and extracellular matrix proteins) that regulate HSC survival, differentiation, expansion, and self-renewal [Friedman et al., 2007; Kassem, 2004; Le Blanc and Pittenger, 2005; Majumdar et al., 1998; Taghizadeh and Sherley, 2009]. MSCs have been shown to secrete interleukin (IL)-1a, IL-6, IL-7, IL-8, IL-11, IL-12, IL-14, IL-15, macrophage colony-stimulating factor (M-CSF), flt-3 ligand (FL), stem-cell factor (SCF), leukemia-inhibiting factor (LIF), granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF) [Friedman et al., 2007; Majumdar et al., 1998]. HSCs are thought to reside along the endosteal surface of the bone in a nurturing environment, quite often referred to as the BM microenvironment or niche. The paracrine factors produced by MSCs, as well as the potential physical cell-to-cell contact, cue and nurture HSC self-renewal, survival, expansion, and differentiation. It is this complex, symbiotic relationship between HSCs and MSCs that make up the hematopoietic system and provides for long-term hematopoiesis for the duration of life. The mechanism and cues that regulate this relationship have been elusive to scientists, thus far, due to multiple, complex pathways and interactions. However, current research aims to further understanding and decipher the specific factors that cue the long-term maintenance, function, and regeneration of the hematopoietic system.

HEMATOPOIETIC TRANSPLANTATIONS

Due to the dynamic, robust regenerative potential of the stem cells found in the BM, transplantations using BM grafts, in both autologous and allogeneic settings, have been taking place for several decades to treat many hematopoietic-related diseases and disorders (e.g., leukemia, thalassemia, aplastic anemia, and sickle cell anemia) [Barker and Wagner, 2003]. Various hematopoietic graft sources have been used to restore hematopoietic function in otherwise diseased BM. In fact, BM grafts were the first transplant source utilized to restore hematopoietic function in ailing patients. The first successful clinical use of BM grafts were reported in 1957, when two patients diagnosed for advanced leukemia were treated with sublethal whole body irradiation, followed by infusion of BM from their identical twins [Thomas et al., 1959]. Both recipients engrafted promptly, although both patients subsequently relapsed. Nonetheless, this landmark study demonstrated, for the first time, clinical feasibility of this approach and laid the groundwork for successful BM

graft transplantations in subsequent years. Although much understanding came from BM grafts, their procurement, typically from the iliac crest of donors, is quite invasive and carries serious risk to an otherwise potentially healthy individual. To alleviate this risk to the donor, another transplant graft source has taken advantage of circulating HSCs in the peripheral blood.

Hematopoietic stem cells from the BM can be mobilized into the bloodstream using multiple daily dosing of G-CSF or GM-CSF. Mechanistically, G-CSF and GM-CSF induce neutrophil proteases in the marrow microenvironment that cleave VCAM-1 (vascular cell adhesion molecule-1; CD106), an event that appears to be mechanistically related to the release of hematopoietic progenitors, capable of repopulating hematopoiesis, into the circulation [Elfenbein and Sackstein, 2004; Imamura et al., 2005; Lapidot and Petit, 2002; Levesque et al., 2001, 2002; Petit et al., 2002]. Interestingly, it has also been shown that chemotherapy induces the same proteolytic environment, as exhibited by G-CSF or GM-CSF [Levesque et al., 2002]. However, it does not appear that G-CSF- or GM-CSF-mediated mobilization of HSCs causes permanent damage to the marrow microenvironment or depletion of HSCs in healthy donors in a manner that would be detrimental to a healthy donor, since BM harvested from G-CSF-treated donors maintain their early engraftment potential. However, the chronic effects of the acute G-CSF or GM-CSF dosing regiment on healthy donors remains uncertain.

On the day of collection, mobilized HSCs in the peripheral blood (referred to as MPB) are collected using apheresis and either transplanted right away (typically in allogeneic transplantations) or cryopreserved for later use (either autologous or allogeneic transplantations). A prospective randomized clinical trial demonstrated that MPB HSCs are superior to unstimulated (unprimed, steady state) BM with respect to speed of initial engraftment. This rapid engraftment translates into reduced aplasia, infection risk, requirements for platelet transfusions, number of overall days in the hospital, and, thus, overall cost to the patient [Elfenbein and Sackstein, 2004; Schmitz et al., 1996].

Interestingly, growth factor-stimulated HSCs seem to be equally potent, whether they are obtained from marrow or from blood [Damiani et al., 1997]. When G-CSF-primed BM autografts are transplanted, they engraft nearly as rapidly as MPB, despite containing 40% fewer CD34+ cells than MPB [Elfenbein and Sackstein, 2004]. CD34 is a cell surface glycoprotein expressed on HSCs, but it is not uniquely expressed on HSCs, as downstream hematopoietic progenitor cells (HPCs; e.g., myeloid and lymphoid progenitors) also express CD34 [Taghizadeh and Sherley, 2009]. Regardless, overall survival at a median follow-up of 3.6 years is essentially identical for patients treated with MPB, unprimed, or G-CSF-primed BM grafts. The days to platelet and granulocyte engraftment are quickest with MPB, compared with G-CSF-primed BM and normal, unprimed BM. This observation corroborates the potential priming effect of G-CSF in promoting maturation of short-term HPCs that result in relatively quicker engraftment [Damiani et al., 1997].

Additionally, growth-factor mediated mobilization of HSCs has demonstrated increased expression of CXCR4 (chemokine CXC motif receptor 4), the chemokine receptor that binds stromal derived factor 1 (SDF-1 or CXCL12). Increased CXCR4 expression results in greater hematopoietic homing capacity in vivo and results in increased hematopoietic engraftment. In fact, when UCB is cotransplanted with UCB-derived MSCs, transduced to constitutively upregulate production of GM-CSF in NOD/SCID (nonobese diabetic/severe combined immunodeficient) mice, a significant increase in hematopoietic engraftment is observed, presumably due to increased UCB homing capacity. These results not only exhibit the importance of increasing homing capabilities of transplanted HSCs, but furthermore signifies the potential role of MSCs—and the factors produced by MSCs—in

enhancing engraftment of transplanted HSCs [Friedman et al., 2007; Han et al., 2007; Majumdar et al., 1998; Taghizadeh et al., 2011a, 2011b].

However, the biggest limitation of BM-derived transplantations (including MPB grafts), beyond donor morbidity and risk, is the lack of suitable human leukocyte antigen (HLA)-matched donors for allogeneic transplantations. Careful matching between donor and recipient HLA genes is a key factor influencing transplantation outcomes. For unrelated donor transplants, the National Marrow Donor Program (NMDP) requires a minimum 6 of 8 HLA matched alleles in HLA-A, -B, -C, and -DR. Due to the lack of suitable HLA matched units, the median time to donor identification is a long 49 days [Barker et al., 2002].

UMBILICAL CORD: SOURCE OF PERINATAL HSCs AND MSCs

Clinically relevant sources of stem cells for hematopoietic transplantation is not limited to adult tissue [Laughlin et al., 2004]. HSCs and MSCs exist in perinatal (afterbirth) tissue, including the UC, placenta, and amnion. HSCs are present in the UCB and placenta; MSCs exist in the UC tissue, placenta, amnion, and amniotic fluid [Migliaccio et al., 2000; Rhodes and Mikkola, 2009; Rubinstein et al., 1998; Sirchia and Rebutta, 1999; Taghizadeh et al., 2011a]. The remainder of this chapter will focus on HSCs and MSCs derived from the UC.

The UCB that flows within the umbilical vessels is a rich source of HSCs. The perinatal HSCs found within the UCB are immunologically naive and exist in greater frequency, relative to BM and MPB. UCB-derived HSCs, like BM- and MPB-HSCs, provide for the long-term memory and maintenance of hematopoiesis. UCB HSCs are easily accessible without any patient risk or morbidity since they are derived from tissue that would otherwise be discarded as medical waste. However, unlike BM, which contains a rich source of both HSCs and MSCs, UCB predominantly contains HSCs and has a limited supply of MSCs [Secco et al., 2008; Taghizadeh et al., 2011a]. The UC, on the other hand, is a rich source of MSCs and a poor source of HSCs [Taghizadeh et al., 2011a].

The UC originates from the yolk sac and allantois and is, therefore, genetically derived from the fetus. The UC forms by the 5th week of fetal development, replacing the yolk sac as the main nutrient source for the developing fetus [Meyer et al., 1978]. The normal UC contains one umbilical vein—supplying oxygenated, nutrient-rich blood to the fetus from the placenta—and two umbilical arteries—clearing deoxygenated, nutrient-depleted blood back to the placenta. The vessels are embedded in a proteoglycan-, collagen-rich matrix, quite often referred to as the Wharton's jelly. This embryonic mucous connective tissue is derived from the extraembryonic mesoderm. Its main role is to prevent the compression, torsion, and bending of the umbilical vessels, which are vital for providing the bidirectional blood flow between the fetal and maternal circulation. UC MSCs are predominantly found within the Wharton's jelly (WJ) and, like BM-derived MSCs, provide for the long-term memory and maintenance of differentiated mesoderm tissue (i.e., bone, cartilage, muscle, and fat). Unlike BM, however, the isolation of MSCs from the UC is noninvasive, will not increase the likelihood of infection, bleeding, or chronic pain to either the newborn or mother [Taghizadeh et al., 2011a].

Cells found within the UC WJ are a primitive MSC, likely trapped in the connective tissue matrix as they migrated to the AGM (aorta-gonad-mesonephros) region through the developing cord during embryogenesis (prior to E10.5) [Wang et al., 2008]. During early embryogenesis, hematopoiesis takes place in the yolk sac and later in the AGM region.

Colonies of early hematopoietic and mesenchymal cells migrate through the early UC to the placenta between embryonic day 4 and 12 of embryogenesis. A second migration takes place from the placenta again through the early UC to the fetal liver and then finally to the fetal BM, where hematopoietic colonies engraft and predominantly reside for the duration of life. Included in these migrating hematopoietic colonies are early precursors of HSCs, as well as primitive MSCs. Scientists have postulated that during this migration to and from the placenta through the UC in early embryogenesis, MSCs become embedded in the Wharton's jelly and remain there for the duration of gestation [Wang et al., 2008].

Mesenchymal stem cells from perinatal UC that formed during the earliest ontogenic period exhibit significant differences in expansion potential compared with (adult) BM MSCs. The number and potency of BM MSCs specifically decreases with age, as indicated by lower *in vitro* CFU-F (colony forming unit—fibroblasts) and proliferative potential, shorter telomeres, longer population doubling times, and shorter times to senescence [Campagnoli et al., 2001; Gotherstrom et al., 2003, 2005; Guillot et al., 2007]. UC MSCs, on the other hand, maintain the same multipotent differentiation potential with relatively higher CFU-F and proliferative potential, longer telomeres, shorter population doubling times, and longer times to senescence, without loss of stem cell potency. Thus, umbilical cord-derived MSCs appear to be more primitive than those found in adult sources (i.e., BM, adipose tissue, and dental pulp) and represent an earlier MSC population [Carlin et al., 2006; Friedman et al., 2007; Taghizadeh et al., 2011a; Troyer and Weiss, 2008].

HEMATOPOIETIC TRANSPLANTATIONS OF UMBILICAL CORD BLOOD

Like adult HSCs derived from BM and MPB, UCB-derived perinatal HSCs provide for hematopoietic engraftment and regeneration. UCB has been used in many autologous and allogeneic hematopoietic transplantations, including for the treatment of acute myelocytic leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), non-Hodgkin lymphoma, Fanconi anemia, severe aplastic anemia, sickle cell anemia, thalassemia, and many more disorders [Barker and Wagner, 2003]. Furthermore, multiple clinical trials have investigated the use of UCB for nonhematopoietic indications, including neuroblastoma, diabetes, cerebral palsy, and traumatic brain injury [Harris, 2009]. Compared with BM, UCB exhibits increased frequency and size of colonies in CFC (colony forming cell) and LTC-IC (long-term culture initiating cell) assays with greater self-renewal capacity, increased engraftment in NOD/SCID mice, and threefold higher SRC (SCID repopulating cell) frequency [De Wynter et al., 1999; Holyoake et al., 1999; Leung et al., 1998; Mayani and Lansdorp, 1998]. Despite having a higher fraction of HSCs relative to BM, patients transplanted with UCB, however, exhibit a relatively longer time to engraftment. This is mainly due to limitations on the collected UCB volume and required UCB TNC (total nucleated cell) transplantation dose for efficacious engraftment. In fact, clinical trials comparing UCB with BM grafts exhibited time-to-neutrophil recovery ranging from 22 to 27 days in patients transplanted with UCB, as compared with 18 days for an unrelated BM transplant cohort [Laughlin et al., 2004; Takahashi et al., 2004]. In a related study, by posttransplantation day 60, 75% of patients transplanted with UCB grafts exhibited engraftment to normal neutrophil levels, compared with 89% of patients transplanted with BM-derived grafts [Rocha et al., 2004]. Additionally, clinical trials comparing UCB and BM graft transplantations in patients with acute and chronic leukemia indicated relatively slower overall engraftment in UCB cohorts. The median overall survival percentage of patients transplanted after 2 years with UCB or BM grafts was 36 or

42%, respectively [Rocha et al., 2004]. However, after 3 years, the median overall percentage survival was observed to be 26 and 35% in UCB and BM transplanted patients, respectively [Laughlin et al., 2004].

The main reason for the observed relative delayed engraftment is the finite cord blood volume that can be procured from the UC vessels. There exists a direct correlation between volume of UCB collected and recovered TNCs, as well as the rate of neutrophil and platelet recovery (i.e., engraftment) and infused TNC dose. Since the volume of each UCB preparation is intrinsically limited, the number of HSCs obtained from UCB collections is finite. At a desired UCB TNC infusion dose of 25–30 million TNCs/kg (total nucleated cells/kilogram) patient weight, currently UCB units are sufficient to engraft recipients weighing on average less than 50 kg (i.e., small children), and single UCB preparations are currently not efficacious for transplanting adults, or even larger children [Locatelli et al., 1999; Migliaccio et al., 2000; Wagner et al., 2002].

Despite these drawbacks, UCB cells are still the focus of intense research, primarily due to their availability, reduced stringency for HLA matching, and higher HSC fraction. In fact, since UCB cells are collected and stored cryogenically, UCB does not have the same donor attrition issues as adult sources of HSCs. The time from unit identification to transplantation is significantly reduced to days-to-weeks for UCB sources, rather than weeks-to-months for MPB or BM sources. Even before cryogenic storage, UCB units are available for transplantation, as they are fully HLA-typed without risking donor attrition or morbidity. The median time to donor identification for UCB units is 13.5 days compared with 49 days for BM [Barker et al., 2002]. The collection of UCB is less invasive than BM aspirates since UCB is collected from tissue that would otherwise be discarded as medical waste. UCB transplanted units result in lower likelihood of graft-versus-host disease (GvHD), in addition to allowing for more permissive HLA matching (3 of 6 HLA allele match), compared with the required 6 of 8 HLA allele match with BM grafts. UCB-derived HSCs also have a greater proliferative capacity, compared with BM or MPB [Barker and Wagner, 2003; Barker et al., 2002].

STRATEGIES TO OVERCOME THE TRANSPLANT-RELATED LIMITATIONS OF UMBILICAL CORD BLOOD

Due to the promising potential of UCB transplantations, various technologies have been investigated—and are under current examination—for increasing the transplant efficiency of UCB. One such exploration aims to expand HSCs *ex vivo* from UCB. Unfortunately, to date, there have been no successful clinical demonstrations of the expansion of long-term HSCs *ex vivo* [Jaroscak et al., 2003; Shpall et al., 2002; Taghizadeh and Sherley, 2009]. This is not due to lack of effort, as there have been over 2300 peer-reviewed publications reported in the past 40 years. Various mechanisms have been investigated to promote the *ex vivo* expansion of HSCs from UCB, such as the regulation of aryl hydrocarbons [Boitano et al., 2010], BMP (bone morphogenic protein), wnt, HOXB4, notch, coculture on MSC monolayers [Hofmeister et al., 2007; Kelly et al., 2009], and sorting/removal of differentiated cells [Csaszar et al., 2012]. Another strategy uses suppression of asymmetric cell kinetics (SACK) by regulating purine nucleotide pools to promote symmetric expansion of self-renewing, asymmetrically cycling long-term HSCs [Lee et al., 2003; Merok and Sherley, 2001; Pare and Sherley, 2006; Sherley, 2002; Taghizadeh and Sherley, 2009]. However, the biggest technical hurdle to *ex vivo* expansion of HSCs has

been the inability to maintain HSC survival *ex vivo* after explant from the body [Taghizadeh and Sherley, 2009].

Despite the generally universal lack of success in expanding HSCs *ex vivo*, several clinical studies have explored transplantation of putative *ex vivo* expanded UCB units, with the primary aim of evaluating safety and efficacy [Jaroscak et al., 2003; Shpall et al., 2002]. In addition, reduction of the commonly encountered posttransplant pancytopenia to achieve decreased transplant-related morbidity (TRM) and time-to-engraftment were evaluated as a secondary aim. The outcome of these studies indicated the expansion of committed HPCs, since expansion of primitive, long-term HSCs were not demonstrated. Furthermore, these clinical trials failed to show better recovery kinetics over historical controls, and the incidence of both acute and chronic GvHD were higher. Moreover, higher TRM was observed, resulting in higher relapse rates and poor overall survival [Devine et al., 2003]. One rationale for these observations can be attributed to the exhaustion of long-term HSCs, due to the inability to maintain HSC survival *ex vivo* [Taghizadeh and Sherley, 2009]. Overall, these studies demonstrate the lack of underlying scientific development in understanding the exact nature of *ex vivo* expansion of HSCs, especially in UCB units.

The lack of sufficient HSCs in UCB can potentially be overcome by transplanting multiple UCB units. However, the likelihood of finding multiple UCB units that meet the minimum criteria for transplantation is extremely low. Minimum criteria include prefreeze and postthaw TNC counts, viability, functional activity via colony forming cell assays, CD34+ cell fraction and, most importantly, suitable HLA matching (e.g., minimum 3 of 6 HLA match). Interestingly, patients infused with multiple UCB units exhibit engraftment by HSCs from only one of the combined units by day 100 [George et al., 2006; Graves et al., 2007; Nauta et al., 2005]. This finding may indicate that a small number of HSCs is sufficient for hematopoietic engraftment in humans (as has been shown for mouse HSCs [Cao et al., 2004; Moore et al., 1997; Takano et al., 2004], or that some units have factors that allow for preferential homing to the BM [Schoemans et al., 2006]. However, the underlying mechanism postulated to account for the modest increase in the engraftment of combined UCB units is the increased total number of infused primitive HSCs in the combined transplant sample. Notwithstanding, the limitation of finding multiple units that meet the minimum criteria for transplantation can be overcome by increasing the number of UCB units that are publicly banked, thus, increasing the chance of finding multiple units that are suitable for allogeneic transplantation [Barker and Wagner, 2003; Barker et al., 2002; Taghizadeh and Sherley, 2009].

Further investigations into increasing the efficiency of HSC engraftments have explored increasing homing capabilities of HSCs from UCB to the BM microenvironment via SDF-1 gradients. Recent evidence suggests that HSCs may be primed for engraftment by several factors, including C3 complement cleavage fragments (C3a), fibronectin, fibrinogen, and hyaluronic acid [Delaney et al., 2010; Reza et al., 2003, 2006; Wysoczynski et al., 2005, 2007, 2009]. However, in essence, the same increased homing capabilities can be achieved using direct intra-femoral injections of the infused graft, rather than the systemic infusions via intravenous transplantations, albeit with greater patient morbidity [Mckenzie et al., 2005]. The efficacy of increasing homing capacity by chemotaxis is currently under further rigorous investigation.

An additional approach to increase the engraftment efficiency of UCB—to the levels observed with BM—rests on the principle that UCB lacks a significant population of MSCs [Friedman et al., 2007; Secco et al., 2008; Taghizadeh et al., 2011a]. As previously discussed, in the BM, MSCs produce and secrete various factors that provide support and promote mitotic activity, survival, expansion, differentiation, self-renewal of HSCs, as well

as maintaining multilineage differentiation of mesodermal cells. The simple absence of significant numbers of MSCs in UCB may be the biological key in increasing the engraftment efficiency and potential use of UCB-derived HSCs without the dependence of any of the aforementioned *ex vivo* manipulation technologies or multiple unit infusions. Although a significant population of MSCs are absent from UCB, the cytokines and factors that are produced and secreted by MSCs are visible to HSCs in the UCB, since MSCs exist in the tissue surrounding the vessels within the UC (i.e., Wharton's jelly). It is hypothesized that these factors produced by MSCs in the Wharton's jelly are available to the UCB *in utero* primarily by way of diffusion from the WJ to the blood. Additionally, MSCs exist in the placenta and amnion and provide for further local UCB maintenance [Taghizadeh et al., 2011a].

To take advantage of the paracrine factors secreted by MSCs and their functional effects on UCB HSCs, currently, there is great interest and effort to bank and cryopreserve native MSC populations from the Wharton's jelly of UC tissue. Native MSCs are primary MSCs residing within the UC, procured without *ex vivo* manipulation and expansion. Native MSCs derived from UC tissue can be thawed and potentially used to increase engraftment potential of UCB units. In fact, several groups have investigated the *in vivo* engraftment effects of cotransplanting MSCs from the Wharton's jelly of UC tissue, along with UCB, derived from both native UC MSCs [Taghizadeh et al., 2011b] and *ex vivo* culture expanded UC MSCs [Friedman et al., 2007]. In fact, in preclinical trials, where human UC MSCs are cotransplanted with human UCB into NOD/SCID IL2 receptor gamma null mice, a significant increase in human HSC engraftment is observed [Friedman et al., 2007; Taghizadeh et al., 2011b]. These results suggest that the derived, cryopreserved MSCs maintain postprocessing, postcryopreservation functional activity—for both native MSCs and *ex vivo* expanded UC MSCs—although a greater number of *ex vivo* expanded MSCs were necessary to achieve the same level of engraftment as native UC MSCs. This observation suggests that even though *ex vivo* expansion results in an overall increase in the MSC population, it does so at the expense of MSC potency [Friedman et al., 2007; Taghizadeh et al., 2011a, 2011b].

Clinical trials have exhibited the safety and potential efficacy of MSCs in increasing hematopoietic engraftment. In one clinical trial, autologous BM was procured from 28 breast cancer patients. MSCs were cultured and expanded under GMP (good manufacturing practice) compliance under a Food and Drug Administration (FDA)-approved IND (investigational new drug) application. The autologous BM-derived MSCs were infused into 28 breast cancer patients receiving MPB grafts, in an effort to augment hematopoietic engraftment [Koc et al., 2000]. No toxicity related to the intravenous-infused expanded MSCs were observed. Furthermore, rapid hematopoietic engraftment was observed in all patients, with median neutrophil recovery (>500/ml) in 8 (range: 6–11) days and platelet count recovery >20,000/mL and >50,000 unsupported in 8.5 days (range: 4–19) and 13.5 days (range: 7–44), respectively [Koc et al., 2000]. This study was not designed to determine the efficacy or mechanism of action of the infused MSCs with respect to hematopoietic engraftment. It did, however, justify further clinical studies to investigate the effect of cotransplanted MSCs on the engraftment of hematopoietic grafts, such as UCB, in controlled, randomized, multicenter clinical trials. Additional clinical studies have further established the safety and efficacy of infused MSCs and in the setting of hematopoiesis, have exhibited accelerated engraftment, positive outcomes, and potential treatments for GvHD [Ball et al., 2007; Caimi et al., 2010; Fouillard et al., 2007; Lazarus et al., 2005; Le Blanc et al., 2007, 2008].

Although the exact mechanism(s) of action of MSCs in accelerating hematopoietic engraftment from HSCs from UCB is currently unknown, there are several postulations

regarding the potential mode of action of MSCs in this setting. MSCs may be promoting greater survival of the HSCs during homing, thus, enabling quicker engraftment. With greater HSC survival, more HSCs can reach the BM and subsequently engraft. This is the same mechanism observed in the BM microenvironment and in vitro coculture experiments [Lewis et al., 2001; Moore et al., 1997; Nolta et al., 2002; Punzel et al., 1999]. MSCs may also be increasing the homing capacity of UCB HSCs, directly effecting the sensitivity of the SDF-1 homing receptors (i.e., CXCR4), believed to be responsible for HSC homing and engraftment in the BM [Delaney et al., 2010; Reca et al., 2003, 2006; Wysoczynski et al., 2005, 2007, 2009]. Alternatively, MSCs may be indirectly increasing homing capacity by suppressing the initial, local immune response by the host, as an initial immune response has been observed with UCB injections intravenously (IV) [Pearson et al., 2008; Pino et al., 2010]. Cells that function in innate immunity—specifically natural killer (NK) cells—quickly destroy infused UCB cells transplanted IV in NOD/SCID mice, presumably resulting from residual, “leakiness” of the animal model. However, when UCB cells are transplanted via IV into NOD/SCID IL2R gamma null mice—where no residual NK cells exist, a sixfold increase in hematopoietic engraftment is observed [Pearson et al., 2008; Pino et al., 2010], demonstrating the critical role of the initial host immune response in transplanted allogeneic grafts. MSCs may suppress this initial immune response, thereby enabling greater numbers of viable HSCs to home and engraft into the BM microenvironment [Weiss et al., 2008]. MSCs may also be promoting local expansion of the UCB HSCs, much in the same way that is observed in the BM microenvironment. In fact, there is evidence that cocultured MSCs with UCB HSCs can promote expansion of HSCs [Lewis et al., 2001; Moore et al., 1997; Nolta et al., 2002; Punzel et al., 1999]. However, this *ex vivo* expansion results in significantly less HSC activity when compared with unmanipulated, native HSCs possibly, due to the loss of HSC survival during the *ex vivo* culture expansion [Taghizadeh and Sherley, 2009; Taghizadeh et al., 2011a, 2011b].

Moreover, since many groups have established the safety and efficacy of clinical-scale autologous and allogeneic human MSC expansion and intravenous infusion [Ball et al., 2007; Boomsma et al., 2007; Hare et al., 2009; Koc et al., 2000; Lazarus et al., 1995, 2005], clinical trials are currently being conducted to determine the safety and efficacy of MSCs targeted toward numerous other hematopoietic- and nonhematopoietic-related disease indications. Some of these indications include GvHD, Crohn’s disease, inflammatory bowel disease, and T1D using off-the-shelf BM-derived MSC products that have been manufactured to clinical scale. These studies are designed to exploit the immunosuppressive properties of MSCs. Clinical trials are also investigating the efficacy of MSCs in promoting tissue renewal, regeneration, and wound healing/repair in acute myocardial infarction (AMI), pulmonary disease (PD), congestive heart failure, peripheral vascular disease, peripheral arterial disease, critical limb ischemia, ischemic stroke, traumatic brain injury, multiple sclerosis, Parkinson disease, and even spinal cord injuries [Boozer et al., 2009; Hare et al., 2009; Kebriaei et al., 2009; Mannon, 2011; Mays et al., 2007; Prasad et al., 2011; Van Bokkelen, 2011]. Of course, MSCs are being investigated for potential benefits in arthritis, focal bone regeneration, and sports medicine for harnessing the multilineage mesodermal regeneration capabilities of MSCs.

UMBILICAL CORD TISSUE MSC BANKING

Due to the vast potential clinical applications of MSCs, in both hematopoietic and non-hematopoietic disease indications, MSCs from the UC are now being banked, alongside UCB, for future clinical transplantations. The same native MSCs that are trapped in early

embryonic development during the migration to and from the placenta through the early UC can be easily collected and harvested from the Wharton's jelly of the UC at the time of delivery. This ease of collection has obvious advantages over the collection of adult stem cells from BM and adipose tissue, for which the donor has to undergo an invasive surgical procedure [Taghizadeh et al., 2011a]. This factor, coupled with the great expansion capabilities of UC-derived MSCs, enables this cell source to represent a virtually inexhaustible supply of MSCs for both autologous and allogeneic cellular and regenerative medicine therapies [Schugar et al., 2009; Taghizadeh et al., 2011a; Weiss et al., 2006].

At the time of delivery, after the donor's UCB has been collected, the UC, in its entirety, is clipped, and placed in a collection jar. The collected cord blood and cord are placed in a kit and shipped to the processing center, whereby the UCB is processed using both validated manual and automated methods; the UC is processed using a separate, validated technique, which involves a series of processing and separation steps. The final homogenous cell product from the UC is cryopreserved in a 25-mL cryopreservation bag, similar to the one used for the associated cord blood unit. UC MSCs are then cryopreserved at a controlled rate, and then transferred to liquid nitrogen for long-term storage, once the units have passed all quality controls and are found to be free of pathogens and contaminants. Samples are taken from each unit and characterized for expression of cell surface proteins using flow cytometry, including CD105 (endoglin receptor), CD73 glycoprotein, CD90 (Thy-1), CD44 (homing-associated cell adhesion molecule; H-CAM), CD29 (Integrin b1), HLA-ABC, HLA-DR, CD34, and CD45. Further characterizations may include CFU-F (colony-forming unit-fibroblast), expansion potential, and multilineage differentiation along osteogenic, chondrogenic, and adipogenic lineages [Dominici et al., 2006; Taghizadeh et al., 2011a]. Unit sterility (i.e., lack of bacterial contaminants) and the indicated characterizations are necessary for release of the unit for transplantation in FDA-approved indications.

The processing of UCB is performed such that the innate, native mononuclear cell population is extracted from the whole UCB in a timeframe that would not alter the biological properties of the cells and without any significant *ex vivo* manipulation (e.g., *ex vivo* expansion). This same framework can be used to extract native MSCs from the UC tissue after the collection of the UCB without changing the native biological properties of the derived MSC population. Once the extracted MSCs are collected and cryopreserved, they represent the native MSCs that were once trapped within the Wharton's jelly between day 4 and 12 of embryonic development [Taghizadeh et al., 2011b; Wang et al., 2008]. Once the MSCs are placed into *in vitro* culture flasks or bioreactors, the resultant cell products have biologically changed, in that they no longer represent the same parent population, but are, rather, cell progenies procured under artificial biological conditions (i.e., incubators, T-flask, bioreactors, and medium).

Although, it is the aim of culture expansion to maintain the native properties of the derived MSCs, this is not the case. MSCs are classically defined as cells that will adhere to plastic surfaces, express MSC markers, such as CD90, CD73, CD105, CD44, CD29, lack expression of hematopoietic markers CD45, CD14, CD34, and have the capacity for multilineage differentiation into osteocytes, chondrocytes, and adipocytes [Dominici et al., 2006]. The challenge with this artificial definition is that it defines MSCs prospectively, rather than defining the properties of the actual native population of MSCs *in vivo*. The native MSCs in adipose tissue, dental pulp, UC tissue, or BM, do not possess plastic adherence *in vivo*, although as soon as native MSCs are derived and placed into plastic flasks, they selectively adhere to the coated plastic and expand. Culture expansion of MSCs results in the loss of MSC potency, as native MSC activity is gradually lost (and not replenished) and has been found to lead to accelerated aging and senescence *in vitro*

[Campagnoli et al., 2001; Friedman et al., 2007; Gotherstrom et al., 2005; Guillot et al., 2007; Taghizadeh et al., 2011b]. Culture-expanded UC MSCs exhibit lower rates of hematopoietic engraftment compared with unmanipulated, native UC MSCs—when cotransplanted at the same UCB and UC MSC dose [Friedman et al., 2007; Taghizadeh et al., 2011a, 2011b]. Therefore, it is for these reasons that methods have been developed to collect native HSCs and MSCs from the UCB and tissue, respectively, as these native cell populations possess the most potent stem cell activity. This way the full therapeutic benefit of each respective stem cell population can be attained and harnessed without risk of changing the innate biological properties of each stem cell population.

Both hematopoietic and MSCs maintain great therapeutic potential. HSCs, especially those derived from UCB, are currently being investigated in clinical trials in the treatment of numerous hematopoietic and nonhematopoietic disease indications. Much effort has been placed in showing the safety and efficacy of MSCs in the clinics, especially those derived from BM and adipose tissue. As the field moves forward, additional clinical studies will investigate the safety and efficacy of MSCs derived from the Wharton's jelly of the UC tissue, in not only the setting of accelerating hematopoietic engraftment with UCB, but also in regulating tissue repair/renewal, immunosuppression, and mesodermal tissue formation in the treatment of various diseases from neurodegenerative indications, diabetes, and sports injuries. Certainly, the use of perinatal stem cell populations in cell-based therapies and regenerative medicine is very promising. However, much effort is still needed to bring this promise to clinical fruition for the safe and efficacious treatment of ailing patients.

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