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Placental tissue stem cells and their role in neonatal diseases

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ABSTRACT

Neonatal diseases such as hypoxic ischemic encephalopathy, diseases of prematurity and congenital disorders carry increased morbidity and mortality. Despite technological advancements, their incidence remains largely unabated. Stem cell (SC) interventions are novel therapies in the neonatal world. In pre-clinical models of neonatal diseases, SC applications have shown encouraging results. SC sources vary, with the bone marrow being the most utilized. However, the ability to harvest bone marrow SCs from neonates is limited. Placental-tissue derived SCs (PTSCs), provide an alternative and highly attractive source. Human placentas, the cornerstone of fetal survival, are abundant with such cells. Comparing to adult pools, PTSCs exhibit increased potency, decreased immunogenicity and stronger anti-inflammatory effects. Several types of PTSCs have been identified, with mesenchymal stem cells being the most utilized population. This review will focus on PTSCs and their pre-clinical and clinical applications in neonatology.

1. Introduction

Human placentas, initially considered a medical waste, are increasingly recognized as a source for progenitor cells with vast therapeutic potential. The medical research community took interest in placental tissues as a source for multipotent stem cells (SCs) in the 1980s, after the successful use of umbilical cord blood SCs to treat a five year old with Fanconi's anemia [1]. In the following decades, research into SCs sourced from human placenta and umbilical cord has greatly expanded and gained traction. The establishment of placental and umbilical cord blood banks has supported the initiative and currently there are more than 100 trials in the National Institutes of Health (NIH) registry examinining the therapeutic applications to treat various human diseases.

Extremely premature infants require intensive medical interventions such as mechanical ventilatory support, intravenous parental nutrition, broad spectrum antibiotics and/or inotropic treatments to sustain life due to multi-organ immaturity. A significant number of them will develop severe complications, such as bronchopulmonary dysplasia, necrotizing enterocolitis, and intraventricular hemorrhage which increase morbidity and mortality. These conditions are difficult to manage and carry a large socioeconomic burden [2]. Similarly, genetic conditions that rise in infancy with grave prognosis, as well as perinatal asphyxia [3] require intensive care services that despite technological advancements have poor outcomes. SC therapeutics can lead to a breakthrough in the medical management of these conditions in the ever

Abbreviations: SC, stem cell; PTSC, placental-tissue derived stem cell; MSC, mesenchymal stromal/stem cell; ESC, epithelial stem cell; HPC, hematopoetic progenitor cell; HSC, hematopoetic stem cell; EPC, endothelial progenitor cell; ECFC, endothelial colony forming cell; UC, umbilical cord; UCB, umbilical cord blood; MNC, mononuclear cell; DMSC, decidua mesenchymal stromal/stem cell; CMSC, chorionic mesenchymal stromal/stem cell; AMSC, amniotic membrane mesenchymal stromal/stem cells; AESC, amniotic epithelial stem cell; AFMSC, amniotic fluid membrane mesenchymal stromal/stem cell; WJMSC, Wharton's jelly mesenchymal stromal/stem cell; CPC, cardiac progenitor cell; EV, extracellular vehicles; AC, amnion cell; BPD, bronchopulmonary dysplasia; CDH, congenital diaphragmatic hernia; CHD, congenital heart disease; HLHS, hypoplastic left heart syndrome; HIE, hypoxic ischemic encephalopathy; IVH, intraventricular hemorhage; MMC, meningomyelocele; NEC, necrotizing enterocolitis; TRASCET, transamniotic stem cell therapy.

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expanding field of regenerative medicine.

In this review, we will focus on placental-tissue derived SCs (PTSCs) and their therapeutic application in neonatal diseases. Our aim is to highlight the ongoing research with PTSCs, categorizing the target diseases by organ systems and primary pathophysiology.

2. Stem cell characterisitcs

SCs are defined as un-differentiated cells that have the ability to self-renew and differentiate into specialized cells. Depending on their differentiation capability, they are categorized in potency classes (from high potency to low) such as totipotent, pluripotent, multipotent and unipotent. A totipotent cell, an example of which is the zygote, has the ability to give rise to both embryonic and extra-embryonic structures such as the placenta, yolk sac and allantois. Pluripotent cells give rise to either extra-embryonic or embryonic structures that are formed by the three germ layers (ectoderm, mesoderm, endoderm). Multipotent SCs can differentiate only into cells of a single germ layer, where unipotent cells are those who can differentiate into mature cell types. SCs can be circulating or tissue residents. Depending on the donor, SCs can be subdivided into adult, fetal/perinatal, embryonic or induced SCs. PTSCs belong to perinatal SCs [4].

In the placental tissues the following SC types have been described:

Mesenchymal Stromal Cells (MSCs), are the most featured stem cell population in studies. These cells are multipotent. MSCs, by definition, adhere to plastic under culture conditions, do not express hematopoietic markers and have the ability to differentiate to osteoblasts, chondrocytes and adipocytes. Some placental-tissue derived MSC populations express embryonic markers (Nanog, Oct-4, Sox-2, SSEA-4) [4–6].

Epithelial Stem Cells (ESCs) are traditionally considered an unipotent population with functions directly influenced by the underlying mesenchyme of the organ they reside. However, these cells exhibit pluripotent capabilities if derived from perinatal tissues. Amniotic epithelial SCs (AESCs) can differentiate to all three germ layers. AESCs have been studied for their regenerative abilities through their extracellular vesicles [5,6].

Hematopoietic Stem Cells and Hematopoietic Progenitor Cells (HSCs & HPCs) are unipotent stem cells that can give rise to all mature blood cell lineages [5,7].

Endothelial Progenitor Cells (EPCs) are a heterogenous unipotent group of cells with the ability to migrate and engraft into sites of enthothelial injury, promoting vascular repair and regeneration [8]. EPCs retain the ability to self-renew. Identified by cell culture assays and flow cytometry, EPCs were given a plethora of names. Under the latest nomenclature, only enthothelial colony forming units are recognized as EPCs, as they do not carry hematopoietic markers or give rise to hematopoietic cell lineages [8]. In pre-clinical studies in rodent models of acute lung injury, EPCs have shown great promise to ameliorate the damage caused to the pulmonary endothelium by hyperoxic exposure [9]. EPCs were also found to be effective in rodent models of alveolar capillary dysplasia [10,11].

3. Placental-tissue specific stem cells

The human placenta is the fundamental temporary organ responsible for fetal development and survival. It is the fetomaternal interface for gas, nutrient, hormonal and waste exchange between the mother and the fetus. It is also an immune barrier and its structure can be divided into the decidua basalis, chorion, and amnion with the umbilical cord inserting in the center of the fetal side of the placental disk. The fetal layers comprise of the chorion and amnion, while the decidua represents modified maternal endometrium. Although the placenta consists of multiple cell types, SCs are abundant [12,13]. Based on their location, several SC populations have been identified: (1) Decidua-derived mesenchymal SCs (DMSCs) residing in the decidua basalis; (2) Chorionic mesenchymal SCs (CMSCs) found in the chorion; (3) Amniotic epithelial SCs (AESCs) and Amniotic mesenchymal stromal/stem cells (AMSCs) which are present in the amniotic membranes; (4) Wharton's jelly mesenchymal SCs (WJMSCs) residing in the Wharton's Jelly of the umbilical cord (UC); (5) From the UC blood (UCB) - Hematopoietic progenitor cells, Hematopoietic SCs (UCB–HPCs and UCB-HSCs), Mesenchymal SCs (UCB-MSCs) and Endothelial Colony-Forming Cells/Endothelial Progenitor Cells (UCB-ECFCs/UCB-EPCs); (6) Amniotic fluid mesenchymal SCs (AFMSCs) from the amniotic fluid (Fig. 1). All these populations can be identified by specific surface markers (Table 1).

PTSCs exhibit several advantages compared to peripheral blood or bone marrow derived SCs, such as good immune tolerance, well-established potency to differentiate into tissue specific resident cells, as well as the ability to replenish SC pools [4,6]. PTSCs direct role in

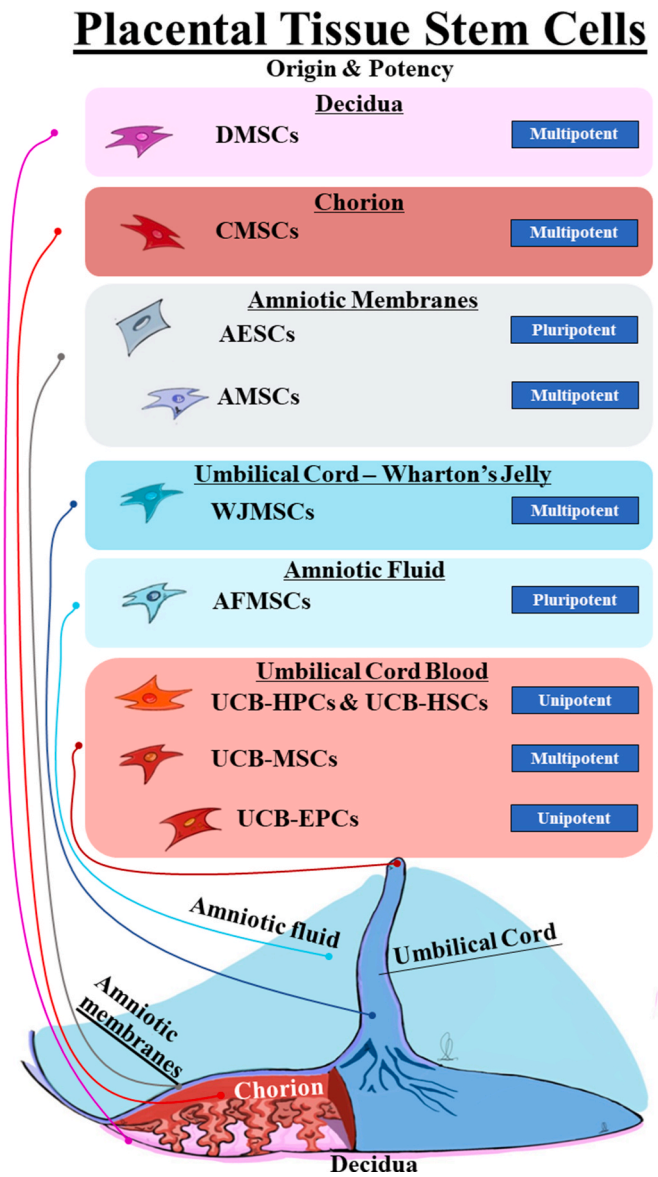

Fig. 1. Placental-Tissue derived Stem Cells based on Origin. Potency in blue boxes. DMSCs: decidual mesenchymal stromal/stem cells, CMSCs: chorionic mesenchymal stromal/stem cells, AESC: amniotic epithelial stem cells, AMSCs: amniotic membrane mesenchymal stromal/stem cells, WJMSCs: Wharton's jelly mesenchymal stromal/stem cells, AFMSCs: amniotic fluid membrane mesenchymal stromal/stem cells, UCB-HPCs and UCB-HSCs: hematopoietic progenitor cells and hematopoietic stem cells respectively, UCB-MSCs: umbilical cord blood mesenchymal stem cells, UCB-EPCs: umbilical cord blood endothelial progenitor cells.

Table 1
Placental tissue stem cells, residency, markers and potency.

Category	Residency	Important Cell Surface Markers	In Vitro Differentiation
Deciduas mesenchymal stromal/stem cells (DMSCs)	Maternal deciduas	CD44, CD73, CD90, CD105, CD146, and CD166 [15]	Multipotent
Chorionic mesenchymal stromal cells (CMSCs)	Fetal chorionic membrane	Similar to AMSCs [5],	Multipotent
Amniotic epithelial stem cells (AESCs)	Fetal amniotic membrane	STRO-1, CD29, CD44, CD49e, CD73, CD90, CD105, CD117, CD166 and ESC marker Nanog, Oct-4, Sox-2, SSEA-4, Tra-1-60 and Tra-1-81 [6,16],	Pluripotent
Amniotic mesenchymal stromal/stem cells (AMSCs)	Fetal amniotic membrane	STRO-1, CD90, CD73, CD105, CD106, CD146, a-SMA, SSEA -3 & −4 [16]	Multipotent
Wharton's jelly mesenchymal stem cells (WJMSCs)	Cushioning matrix between the umbilical blood vessels	Tra-1-60, Tra-1-81, SSEA-1, SSEA-4, TERT, Oct-4, NANOG, and SOX-2 [6, 12]	Multipotent
Amniotic fluid mesenchymal stem cells (AFMSCs)	Amniotic fluid	CD90, CD73, CD105, CD29, CD166, CD49e, CD58, and CD44, Oct-3/4, Nanog, SSEA-4 [16]	Pluripotent potential To all three germ layers
Umbilical cord blood hematopoietic progenitor and stem cells (UCB–HPCs and UCB-HSCs),	Umbilical cord blood	CD34, CD90 AC133,CD117, CD45 [7]	Unipotent; HPCs and HSCs differentiate to myeloid lineages. These populations have been described collectively as Umbilical Cord Blood MonoNuclear Cells (UCB-MNCs)
Umbilical cord blood mesenchymal stem cells (UCB-MSCs)	Umbilical Cord Blood	CD90, CD73, CD105 CD 34 neg, CD 45 neg [17]	Multipotent
Umbilical cord blood Endothelial Progenitor Cells/ Umbilical cord blood Endothelial Colony Forming Cells (UCB-EPCs/ UCB-ECFCs)	Umbilical Cord Blood	KDR/VEGFR2, CD31,CD34, CD133, CD117 [18]	Unipotent; EPCs have the ability to differentiate only to endothelial lineage.

paracrine function is well-established, supporting tissue repair and regeneration, increasing anti-oxidant defenses and inhibiting excessive inflammatory activation. Most importantly, they are usually free of ethical constraints in cases of autotransplantation [4,6,14]. These cells can be sourced from the placenta, UC vessels and WJ, amniotic membranes and even from AF [6,13,14]. The embryonic phenotype that many of these cells exhibit, along with the abovementioned properties have placed PTSCs at the top of potential cell therapies for neonatal diseases.

4. Stem cells and neonatal diseases

4.1. Pulmonary disorders

4.1.1. Bronchopulmonary dysplasia (BPD)

BPD is a syndrome of the premature infants, characterized by lung maldevelopment with alveolar simplification and pulmonary vascular remodeling [19,20]. It is multifactorial and affects up to 40% of extremely premature infants [19,21]. Once established, it is very difficult to manage leading to increased morbidity and mortality. Despite technological advances, the incidence remains relative stable [20]. SC-based therapies are a promising approach to treat this deleterious syndrome.

There are a number of pre-clinical studies evaluating PTSCs in animal models of BPD. Experimental BPD is a lung injury model traditionally caused by hyperoxic and/or ventilatory exposure (through for e.g. baro/volutrauma), via lipopolysaccharide (LPS) administration, or a combination of the above [22,23]. These stressors cause a lung phenotype consistent with alveolar simplification, vascular remodeling and variable degree of fibrosis [22].

Most experimental models of BPD utilize MSCs from the UCB as the preferred source, followed by WJMSCs and placental mesenchymal SCs (PMSCs). Most studies show transient engraftment, while early administration from the stressor insult is better than late in terms of phenotypic outcome [6,24,25]. Routes of administration vary, such as intratracheal, intranasal, intravenous or intraperitoneal [26]. Few studies compare optimal administration route for therapy. Liu at el., found that the intraperitoneal route is superior to the intranasal administration, in a rodent model of BPD [27].

PTSCs improved alveolarization, vascularization and ameliorated the fibrotic and inflammatory phenotype compared to the control groups [6,24,25]. From the assessed mechanisms-of-action, PTSCs decreased activation of the sonic hedgehog pathway [28], inhibited inflammatory cytokine cascades [6,24,25] altered vascular endothelial growth factor (VEGF) levels and decreased elastin levels [28–30]. UCB-ECFCs and amnion epithelial cells (AECs) have had similar effects [31,32]. Studies have evaluated the paracrine role of these cells, via extracellular vehicles (EV), reporting the beneficial effects of the intervention in hyperoxic rodent models of BPD [33,34].

PTSCs have been introduced in clinical trials for BPD. We identified 24 registered trials utilizing PTSCs, out of 261 trials, targeting BPD in the United States National Institutes of Health database (clinicaltrials.gov). Most trials involve UCB-MSCs or UCB Mononuclear Cells (UCB-MNCs). UCB-MNCs are defined by most studies as a heterogenous group of stem cells that are $CD34^{+}$. The majority are phase I trials. From the completed trials using UCB-MSCs, a phase I study of 9 extremely low birth weight neonates, done in Korea, demostrated that UCB-MSCs are safe to administer in two different intratracheal dosing regiments [35]. These neonates were followed for two years and the investigators found no adverse effects linked to the cell administration (NCT01632475) [36]. The same investigators continued with a Phase II double-blinded, randomized, placebo-controlled clinical trial where they saw a significant reduction of severe BPD in neonates born at 23 and 24 weeks but not in older gestational ages who received the UCB-MSCs (NCT01828957) [37]. There is another ongoing phase II trial focusing on the former population (NCT03392467). A similar study from the U.S.A. evaluated two different dosing groups in a Phase I trial of UCB-MSCs, intratracheally administered, in 12 ELBW infants. The study found that both dosing regiments were safe to administer (NCT02381366) [38]. Lim et al. completed a Phase I trial using human allogeneic amnion cells (AC; amnion cells which are AESCs), in six premature neonates with BPD. This trial demonstrated that AESCs can be safely infused [39]. Malhotra et al. followed up the same babies over a two year period and reported that the long term outcomes are reassuring, reinforcing the safety of the cell therapies [40]. Ongoing studies include multiple Phase I trials from China assessing safety and dosing of UCMSCs administered

intratracheally or intravenously (NCT03683953, NCT03873506, NCT03645525, NCT03558334, NCT03378063, NCT03774537). There are also a Phase II trial and two Phase III trials assessing UCB-MNCs, administered intravenously (NCT02999373, NCT05071638, NCT04440670). Baker et al. is evaluating, in a Phase I trial, ACs in 24 infants born at less than 29 weeks of gestation, for the prevention of BPD. From different countries, we identified two studies from Taiwan (NCT03631420, NCT01207869), a study in Vietnam (NCT04062136), Canada (NCT04255147) and Spain (NCT02443961), all assessing a limited number of patients being administered with UCB-MSCs.

4.1.2. Congenital diaphragmatic hernia (CDH)

CDH is a neonatal disorder affecting approximately one in 3000 births. The hallmark of CDH is pulmonary hypoplasia of variable degree and pulmonary hypertension due to compression by the herniation of abdominal organs into the thoracic cavity [41]. Pre-clinical models of CDH include either a surgically-created opening in the diaphragm in order for the abdominal visceral organs to occupy the thoracic cavity or administration of nitrofen to induce a pulmonary and diaphragmatic hypoplasia phenotype [41]. Investigators have used SC therapies to improve the severe phenotype of pulmonary hypoplasia in these models.

Chalphin et al. evaluated transamniotic infusion of AFMSCs in rodents and revealed that the intervention was associated with a significant decrease in arterial wall thickness, endothelial nitric oxide synthase activity and downregulation of fibroblast growth factor (FGF)-10 and VEGF-A gene expressions compared to the untreated group [42,43]. Antounians et al. showed that fetal lung underdevelopment is rescued by the action of the extracellular vesicles (EVs) secreted by amniotic fluid stem cells (AFSCs) in an experimental model of pulmonary hypoplasia [44]. Tzanetakis et al. reported that lung epithelial cells from a rodent model of CDH have elevated endoplasmic reticulum stress and increased apoptosis. The cells were rescued by co-culturing with rodent AFSCs. The investigators concluded that AFSCs exert their action through a paracrine mechanism, a finding that has been reinforced by various investigators using AFSCs in different models of tissue repair [45].

There is one Phase I clinical trial, currently in the recruitment phase, to evaluate the safety profile of UCB-MNCs for hypoxic neurologic injury in infants with CDH. This single center study in Texas, will evaluate 20 participants and follow their two year neurodevelopmental status. (NCT03526588).

4.2. Cardiovascular diseases

Most SC therapies aim to alleviate adult diseases of the cardiovascular system. There is a number of pre-clinical models and clinical trials aimed at the neonatal/pediatric world, many of which assess a specific cell population called cardiac progenitor cell (CPC) and its role in congenital heart disease (CHD). CPCs are progenitor cells native to the heart and are usually harvested during cardiac repair, thus limiting their availability [46]. Several researchers are evaluating the in-vitro differentiation of PTSCs to CPCs. Ali et al. differentiated UCB-MSCs into cells with a myogenic cardiac phenotype that improved the systolic cardiac function in a rat model of myocardial ischemia [47]. Qian et al. differentiated UCB-MSCs into cardiomyocytes using 5-Azacitidine [48], while Lupu et al. utilized UCB-EPCs to reveal that the interaction with living cardiac tissue leads to neovascularization [49]. The studies that use PTSCs directly for CHD are limited. Among the few, Schmidt et al. embedded AFSCs into a biodegradable polymer mesh and fabricated human heart valves [50]. The authors were able to prove ex-vivo that these valves could function in a low pressure/strain environment such as the right atrium and ventricle. Yerebekan et al. used UCB-MNCs to improve the systolic function of the right ventricle in a swine model of Tetralogy of Fallot [51]. Preferred routes of administration were the intracoronary or intramyocardial, with intracoronary administration being at most risk for vascular occlusion [52].

In a phase I clinical trial Burkhart et al. evaluated the safety of UCB-MNCs administration, in 10 infants undergoing Stage II palliative repair of hypoplastic left heart syndrome (HLHS). The team, reported the administration is safe and feasible [53]. There is a Phase II multicenter clinical trial in the recruiting phase, assessing the safety and therapeutic role of UCB-MNCs upon intramyocardial administration in 100 participants with HLHS during stage II palliation (NCT03779711). A phase I study is recruiting from the same team, to include 30 participants in order to evaluate safety of UCB-MNCs intramyocardial administration during Stage III palliation (NCT04907526). Another Phase I trial, in Australia, is recruiting 12 participants to evaluate the safety of autologous UCB-MNCs (NCT03431480).

4.3. Neurological diseases

4.3.1. Hypoxic ischemic encephalopathy (HIE)

HIE is a complication of perinatal asphyxia and is characterized by encephalopathy caused by a period of hypoxia/anoxia and/or hypoperfusion of the fetal/neonatal brain. Around 0.5 to 1 in 1000 neonates are affected [54] and among those, approximately 25% die, while up to 20% of those that survive, have poor neurodevelopmental outcomes [55]. Therapeutic hypothermia is the gold standard therapeutic modality when applied in a timely manner, depending on the severity of the encephalopathy. The intervention is associated with a significant reduction in mortality and developmental disability [54].

With their immunomodulating, antioxidant and repairing properties, SC therapies have a promising role to ameliorate the damage caused by the hypoxic-ischemic insult. Pre-clinical models of HIE based on brain hypoxia/anoxia and/or hypoperfusion due to carotid ligation [56] have been used to rescue the phenotypes by stem cell administration. WJMSCs, UCB-MSCs, UCB-EPCs and PMSCs have been used in models of HIE [57,58]. Intraventricular, intravascular or intranasal administration routes are commonly utilized [57]. The projects utilizing PTMSCs showed reduction in pro-inflammatory cytokines, neuronal apoptosis, inhibition of microglial and astrocytic activation [57,58]. McDonald et al., revealed that MSCs were responsible for increasing the number of hippocampal neurons [59]. However, most studies show transient or no engraftment of MSCs into the brain parenchyma [57]. Only a limited number of studies revealed that SC interventions increased the number of neurons [60,61]. A number of studies investigated the effects of the PTSC secretome on the brain, reporting anti-inflammatory and anti-apoptotic effects, improved neuronal repair and angiogenesis [58]. Of special note, EPCs had the ability to improve cerebral blood flow in addition to the benefits of tissue repair [61].

Cotten et al. completed a phase I trial proving the safety and feasibility of autologous UCB-MNCs therapy to neonates with HIE (NCT00593242) [3]. In 2020, the team completed a multicenter, randomized controlled Phase II trial, to assess safety, efficacy and long term outcomes of UCB-MNCs infusion in neonates with HIE. As of the writing of this review, results of the trial have not been published (NCT02612155). Cotten et al. also completed another study using WJMSCs where the investigators concluded that the cell preparation can be safely administered but further safety follow up studies are warranted (NCT03635450) [62]. Tsuji et al. administered autologous $CD34^{+}$ UCMNCs in HIE neonates, in a phase I trial, reinforcing the safety of the intervention (NCT02256618) [63]. On clinicaltrials.gov there are a number of registered trials that are in the progress of recruiting in Australia, China, France, India and Spain evaluating the effect of PTSCs in HIE (Australia - ACTRN12610000421033, China - NCT02551003, NCT03352310, ChiCTR-TNRC-11001591, ChiCTR-TRC-10000922, France - NCT01649648, NCT02881970, India - NCT03657394, Spain - NCT01506258).

4.3.2. Intraventricular hemorrhage (IVH)

IVH is a serious complication of prematurity which involves germinal matrix hemorrhage into the lateral ventricles increasing the risk of longterm neurodevelopmental impairment in these infants [64,

65]. The more premature the neonate, the greater the risk for IVH. Different animal models of IVH have been used. In preterm rabbits, IVH can be induced by intraperitoneal glycerol administration, whereas intraventricular infusion of blood or collagenase is used to cause IVH in rodents [66]. These models have a variable degree of success in creating an IVH phenotype leading to posthemorrhagic hydrocephalus [66]. Stem cells have been utilized in preclinical models of IVH with UCB-MSCs being the primary cell population studied. Similar to models of HIE, UCB-MSCs show anti-inflammatory, anti-apoptotic, anti-microglial and astrocytic activation, leading to improved phenotype and in some cases, preventing the development of posthemorrhagic hydrocephalus. In these studies SCs were administered via intraventricular, intrathecal, intravenous, intraperitoneal, or intranasal routes. Even though the intranasal route is the least invasive method, it does not offer the same degree of cellular engraftment as the intravenous route. Intraventricular administration revealed the greatest potential for engraftment compared to the intravenous route but the long-term engraftment is limited [66].

From the same team that brought the Pneumostem cells® (a patented UCB-MSC derived population) in clinical trials of neonates with BPD, there are two clinical trials for neonates with severe IVH; a completed phase I trial which reported that the SCs are safe to administer via the intraventricular route (NCT02274428) [67] and a currently active follow up study assessing the long term safety of these cells in this population (NCT02673788).

4.3.3. Neural tube defects/spina Bifida/Meningomyeloceles(MMC)

PTSCs have been also utilized for neural tube defects mainly with the use of transamniotic SC therapy (TRASCET) administration in-utero. Wang et al. has utilized PTMSCs in an experimental model for the treatment of spina bifida [68]. There is currently one active trial, recruiting participants at UC Davis evaluating the safety and role of PTMSCs in prenatal or postnatal MMC repair (NCT04652908).

4.4. Gastrointestinal disorders

4.4.1. Necrotizing enterocolitis (NEC)

NEC is a life-threatening gastrointestinal complication of prematurity and multifactorial in etiology. In its severe form, it is characterized by intestinal necrosis with micro and/or macroperforation, presenting as sepsis and shock. Mortality remains 20–30% of the affected infants [69].

Pre-clinical models of NEC utilize newborn rodents that undergo hypoxic and/or hypothermic exposure with combination of formula feeds with or without in-utero LPS administration [70]. A systematic review and metanalysis by Villamor-Martinez et al., identified nine rodent studies evaluating the role of stem cell therapies in NEC. Six out of these studies utilized PTMSCs with the AFSCs and their exosomes being the most commonly utilized population. The analysis found that SC based therapies reduce the incidence of NEC, improving short term survival. The SCs improved intestinal motility and reduced permeability [70]. There were no clinical trials identified using PTSCs in NEC.

4.4.2. Abdominal wall defects/gastroschisis

Experimental gastroschisis has been the target of PTSC therapy, mainly with the use of TRASCET administration in-utero [71]. Pre-clinical models of gastroschisis involve surgical separation of the abdominal wall in order for the abdominal contents to herniate, mimicking gastroschisis. Feng et al. in a rodent and rabbit experimental models of gastroschisis, found that intra-amniotic administration of AFMSCs led to thicker muscularis and serosal layers compared to the placebo. The authors concluded that AFMSCs improved, but did not prevent, the gastroschisis phenotype [72,73]. Chalphin et al. compared PMSCs to AFMSCs in an experimental model of gastroschisis, demonstrating that while both SC groups managed to mitigate bowel damage, the AFMSCs were found superior in outcomes [74]. No clinical trials have been reported for gastroschisis utilizing PTSCs.

5. Tissue bioengineering focused on pulmonary tissues

Pulmonary tissues bioengineering utilizes a combination of three-dimensional (3D) scaffolds, cellular engraftments and medications to create a functioning, living tissue. These scaffolds are biosynthetic constructs or de-cellularized tissues, such as whole lungs, enriched at a second stage with cell populations. These artificial matrices are directly transplanted into living organisms in order to promote re-cellularization and transformation into functional lung tissue. SCs are an ideal population for such steps given their ability to proliferate and differentiate into mature cell lines. The purpose of the scaffold is to guide and support this differentiation. Pre-clinical studies in rodent models of bioengineered lung constructs, demonstrated that after implantation, the constructs were re-cellularized with airway and gas exchange epithelia, perfused by functioning blood vessels [75–77].

MSCs and EPCs isolated from different sources such as placenta, AF and/or UC have been investigated for their applicability to repopulate these acellular lung matrices. After seeding on de-cellularized lung biological scaffolds, the cells were able to differentiate into functioning respiratory epithelial and vascular units [78]. Surgical implantation of de-cellularized lungs which have been re-cellularized with a mix of fetal lung homogenates show short-term survival and some degree of functionality in in-vivo rodent models. Even though lung tissue bioengineering is still in its infancy, these studies are very promising as they offer future alternatives to lung transplantation, which are compounded by shortages of donor lungs and lifetime immunosuppression [76,79]. In another approach, lung tissue bioengineers have utilized embryonic stem cells infused into mice blastocysts to create in-vivo chimeric lungs [80].

6. Conclusion and future directions

Regenerative medicine has made great advancements with the utilization of SCs to control and improve pathophysiologies of previously-thought uncurable and debilitating diseases. SC therapies for neonatal diseases have been found safe to administer, to date. PTSCs have significant advantages compared to SCs from other sources, as they can be harvested from discarded tissues in plentiful quantities and most importantly, this can happen in a non-invasive manner. Their “stemness” and plasticity remains superior to adult sources. Premature infants can receive their own autologous SCs as therapies for the diseases of prematurity. From all the above, placental tissues could become the best SC source for treatments of refractory human diseases in the future.

Placental tissue SC therapies cannot be the panacea, as there are so many aspects of stem cell biology that remain unknown. While animal models of neonatal diseases are not ideal, they do provide a stepping stone to further SC applications in humans and enhance our understanding of SC behavior. While most studies reveal the beneficial effects of SC therapies, there is the concern for rare complications such as tumorigenesis or infarction by cell masses. This is an important consideration, since stem cells self-renew, and potentially can form tumors. To date, all clinical studies involving PTSCs have proven safe; however further follow up is warranted.

Placental tissue SCs are important also in tissue bioengineering. With the advent of innovative nanomaterials that can serve as scaffolds, SCs can provide the cellular source for tissue generation. This has important implications in organ transplantations. Additionally, the SC secretome is vast and greatly unexplored. Even though, most studies evaluating EVs reveal a beneficial effect to combat diseases, identifying particular secretomes is crucial. It is without a doubt that SCs have opened a door into a plethora of therapeutic applications, yet further identification and refinement are of outmost importance. Judicious use of SC based therapies is critical.

7. Practice points

- **PTSCs** compared to adult SCs have:
  - increased potency,
  - decreased immunogenicity,
  - stronger anti-inflammatory effects,
  - abundant
- Clinical studies reinforce safety of intervention in Neonatal Diseases

Research directions

- Isolation of the most optimal PTSC population for treatment of neonatal diseases
- Identification of the optimal PTSC "dose" for therapy
- Long term follow up of PTSC interventions into adulthood

Declaration of competing interest

No conflict of interest, financial or otherwise, are declared by the authors.

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