Mesenchymal Stem Cells Derived from Wharton's Jelly and their Potential for Cardio-Vascular Tissue Engineering

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Abstract: Experimental results accumulated during last decade suggest that human perinatal tissues such as placenta, fetal membranes, and umbilical cord, as well as perinatal fluids such as, amniotic fluid and umbilical cord blood, harbour different amounts of multipotent precursor cells, called extra-embryonic mesenchymal stem cells (EE-MSCs). Perinatal EE-MSCs represent an intermediate cell type between pluripotent embryonic stem cells (ESCs) and multipotent MSCs derived from variety of postnatal human tissues, such as bone marrow, fat, dental pulp, etc. Multipotent mesenchymal cells obtained from connective Wharton's Jelly tissue of umbilical cord (WJ-MSCs) currently emerged as particularly interesting type of perinatal EE-MSCs, related for therapeutical applications and cryobanking. These cells are easily assessable for isolation, possess fetal karyotype, and hold very active growth potential. Due to their unique developmental position WJ-MSCs exhibit specific phenotype which combines some markers expressed by postnatal bone marrow-derived MSCs, such as CD73, CD90, and CD105 with some markers typically expressed by ESCs, such as Oct-3/4, Sox-2, and Nanog. In terms of plasticity WJ-MSCs demonstrate the potential for differentiation towards mesodermal and ectodermal lineages *in vitro* and *in vivo*. Additionally, extraembryonic tissues are normally discarded after birth and the isolation of WJ-MSCs is free of ethical concerns. In this work we aim to review previously obtained experimental results, discuss different aspects concerning plasticity and immunomodulatory characteristics of WJ-MSCs, and evaluate the potential of these cells for biomedical and clinical applications.

Keywords: Mesenchymal stem cells, wharton's jelly, tissue engineering.

EXTRAEMBRYONIC PERINATAL TISSUES – NEW PUTATIVE SOURCES OF STEM CELLS

Emerging evidence indicates that different postnatal human tissues harbour small amounts of mesenchymal stem cells (MSCs), which have the potential to give rise to all cell types of the source tissue [1, 2]. Previously isolated from human bone marrow, currently MSC harvest sites include the muscles, fat, brain, cartilage, and dental pulp [3-5].

Clinical expectations associated with MSCs are derived from three functional characteristics of these cells: the ability (I) for tissue reparation through direct or paracrine effects, (II) for immunomodulation, and (III) to support cell engraftment. Different research efforts have demonstrated that adult MSCs have a broad therapeutic potential primarily due to their ability to regenerate tissue by differentiating towards multiple adult cell types under appropriate *in vivo* and *in vitro* conditions [6, 7]. Moreover, a growing body of data demonstrates the potential use of *ex vivo*-expanded adult MSCs in modulating immunologic response through interactions with immune cells such as T- and B-lymphocytes, natural killer cells, and dendritic cells [8-11]. These immunosuppressive properties of MSCs characterize them as promising candidates for treating immunologic disorders like Crohn's disease. Additionally, there is increased potential for

MSC use in decreasing occurrences of Graft-versus-Host disease after allogenic haematopoietic stem cell (HSC) and solid organ-transplantation [12, 13].

Earlier source tissues of stem cells were classified into two general categories dependent on the timepoint of onto-genesis: embryonic and postnatal also called adult tissues. In the last decade, the list of putative human stem cell sources was amended to include human perinatal extra-embryonic tissues [14, 15]. Generally, human extra-embryonic tissues are represented by different parts of the placenta, fetal membranes (amnion and chorion), and umbilical cord [16-18]. Furthermore, it has been shown that extra-embryonic MSCs can be isolated from umbilical cord blood (UCB) and amniotic fluid (AF) [19-21].

Extra-embryonic perinatal MSCs represent an intermediate stem cell type that partially combines some pluripotent properties of embryonic stem cells (ESCs) with some multipotent properties of adult postnatal mesenchymal stem cells (MSCs) [15]. Due to its close ontogenetic relationship with embryonic stem cells, extra-embryonic tissue-derived MSCs have immunoprivileged characteristics, possess a broader multipotent plasticity, and proliferate faster than adult postnatal MSCs [14, 15]. Moreover, because extra-embryonic tissues are normally discarded after birth, these cells could be isolated while effectively avoiding ethical concerns [14].

Previously, cells obtained from cord blood, amniotic fluid, and fetal membranes were used in perinatal medicine for invasive diagnostic purposes, such as sex determination,

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or for the detection of fetal infections, rare metabolic disorders, and genetic diseases such as chorioamnionitis, osteogenesis imperfecta, and trisomy-21. The use of peri-natal stem cells in treating haematological and metabolic diseases dates back to the 1980s, when umbilical cord blood was successfully conducted to treat a patient with Fanconi anemia using UCB from human leukocyte antigen (HLA) matched sibling donor [22]. Since then, there have been over 10.000 successful UCB transplants conducted worldwide [23]. The 1990s demonstrated the usability of perinatal extra-embryonic MSCs in tissue engineering applications, just after Langer's and Vacanti brothers' introduction of the concept of regenerative medicine [24, 25].

HUMAN PLACENTAL ANATOMY AND FUNCTION

Functionally, the human placenta can be seen as a foeto-maternal organ that segregates maternal and foetal circulation while serving as a protective immunological barrier and site of oxygen, nutrient, and metabolite exchange [26-28]. It also has multiple endocrine functions and participates in the production of steroid and protein hormones, such as progesterone and estrogen as well as placental lactogen and chorionic gonadotropin [27, 28]. Progesterone is responsible for the maintenance of endometrial lining during pregnancy and prevention of preterm labor by reducing of myometrial contractions [27, 28]. Estrogen is responsible for stimulation of uterine growth to accommodate growing fetus [27, 28]. Placental lactogen regulates maternal glucose, fat, and protein levels, so that it is always available for the growing fetus [27, 28]. Chorionic gonadotropin is reduces maternal immunologic response and protecting placenta against rejection [27, 28].

Anatomically, the human placenta at full term represents a circular discus-like organ, with an average diameter of about 22 cm, thickness of 2.5 cm, and weight of 470 g [27-29]. Structurally, it can be divided into foetal and maternal parts, termed *surfaces*. These surfaces merge and form the smooth chorion as well as the foetal membranes. A chorionic plate with an inserted umbilical cord characterizes the foetal surface. This chorionic plate is covered by amnion, which is composed of a single epithelial layer and an avascular amniotic mesenchyme that is weakly attached to chorionic mesenchyme. The chorionic mesenchyme contains vessels that are linked with umbilical cord vessels on one side and villous tree vessels and veins on another side [27-30].

The maternal surface, termed the *basal plate*, is typified by a sizable extracellular matrix and fibrinoids. It consists of a mixture of fetal extra-villous trophoblastic cells with maternal cells of the uterine decidua, such as decidual stromal cells, natural killer cells, macrophages, and other immune cell types [30]. The basal plate of a full-term placenta is subdivided into 10 to 40 slightly elevated regions called maternal lobes. Maternal lobe location corresponds with the location of 60 to 70 foetal lobes [27-30].

The umbilical cord is an elastic cord connecting the foetus and placenta during pregnancy. Functionally, the cord protects enclosed vessels from compression, torsion, and bending while providing a bidirectional, foeto-maternal blood circulation (Fig. **1**). A mature umbilical cord has a mean length of 50 to 70 cm (though the range spans 25 to 100 cm), *in vivo* diameter of 12 mm, and weight of 100g (ranging from 40 to 150 g) [31, 32].

Fig. (1). Anatomy of the normal human umbilical cord (*macroscopic image*).

Anatomically, the umbilical cord consists of two umbilical arteries and one umbilical vein, both embedded within a specific mucous proteoglycan-rich matrix, known as Wharton's jelly, which is then covered by amniotic epithelium (Fig. **2**). The jelly-like connective tissue is composed of collagen fibres of Type I and III and a glycosaminoglycan (GAG) - containing jelly-like intercellular substance. The collagen creates a three-dimensional network and is in loose contact with amorphic intercellular substance contains mucopolysaccharids, such as hyaluronic acid, and carbohydrates with glycosyl and mannosyl terminals and acid groups [33, 34].
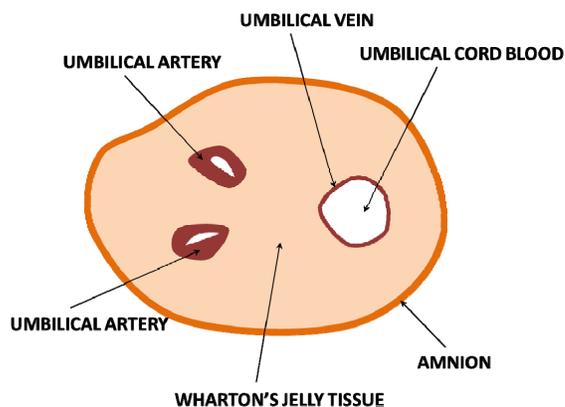
Fig. (2). Structure of umbilical cord blood (*schematic image*).

THE UMBILICAL CORD IS A SOURCE OF MESENCHYMAL STEM CELLS

Results showing that Wharton's jelly contains a multipotent fibroblast-like mesenchymal cell population were first obtained more than ten years ago [35]. Previously, these cells were termed as "umbilical cord matrix stem cells" to distinguish them from endothelial cells isolated from umbilical vein (HUVEC) as well as from late outgrowth

endothelial cells (OECs) and mesenchymal cells (UCB-MSCs) isolated from umbilical cord blood [19, 36, 37]. Though they have lately been termed *Wharton's jelly-derived mesenchymal stem cells* (WJ-MSCs) [38-40].

Currently, WJ-MSCs are isolated from three regions: the perivascular zone, the inter-vascular zone, and the sub-amnion [41]. Fine structural, immuno-histochemical, and functional analysis performed *in vitro* show significant differences in the number and nature of cells among sub-amniotic, inter-vascular, and perivascular regions [42, 43]. These findings lead to hypothesis that these regions might be originating from different pre-existing formations [44]. At the same time it is still unknown whether fibroblast-like cells obtained from different compartments of umbilical cords represents different populations and possess different properties [42]. They express similar surface markers, suggesting that they are all of MSC origin [41]. However, it has been shown that the WJ-MSCs located close to amniotic surface retaining better ability to proliferate, whereas WJ-MSCs with broader differentiation potential were found in closer proximity to the umbilical vessels [42, 43].

The most common methods for WJ-MSC isolation are based on the use of explant-culture or enzymatic digestion techniques [45, 46]. Normally freshly obliterated 15-20 cm umbilical cords will be immediately transported in the laboratory and the umbilical arteries and veins will be removed. After that Whartons's jelly tissue will be excised and minced using a scalpel or surgical scissors, after which the small pieces of tissue will be either directly plated on a tissue culture polystyrene petri dish as explants or additionally digested using proteolytic enzymes (such as collagenase and hyaluronidase). If a digestion step was performed, cell suspensions will be normally filtered through 70-100 um sieves, resuspended in culture medium, and plated out in culture flasks [41, 47]. The advantage of enzymatic digestion technique is related with the purity of obtained cell culture. Normally, if digestion step has been performed, the risk of WJ-MSC contamination with other cell types, such as blood cells or endothelial cells, as well as tissue debris is reduced [41]. Freshly isolated WJ-MSCs normally demonstrate fibroblast-like appearance during the first culture period (10-15 days) until first passage (Fig. **3**). Population doubling time which is approximately 60-85 hours at early passages dramatically declines at late passages [42].

On one hand, WJ-MSCs meet the minimal criteria for defining adult MSCs – morphologically, they resemble plastic adherent adult postnatal MSCs, which are able to self-renew, and can be expanded as an *in vitro* culture [48]. Phenotypically, WJ-MSCs express typical mesenchymal stem cell markers like CD10, CD13, CD29, CD44, CD73, CD90, and CD105. At the same time, they do not express markers of the hematopoietic lineage, such as CD34, and CD45 [49, 50]. In terms of plasticity, WJ-MSCs are multi-potent and can be induced to form adipose tissue, bone, cartilage, skeletal muscle cells, cardiomyocyte-like cells, and neural cells [41, 44].

In another regard, WJ-MSCs possess the typical properties of extra-embryonic perinatal MSCs. Generally properties of WJ-MSCs are very similar with the properties of placenta-derived (PD-MSCs) and properties of cord blood-derived (UCB-MSCs) [16, 19]. First, similarly to PD-MSCs and UCB-MSCs, WJ-MSCs are of mesenchymal origin and possess multipotent plasticity. They have a greater
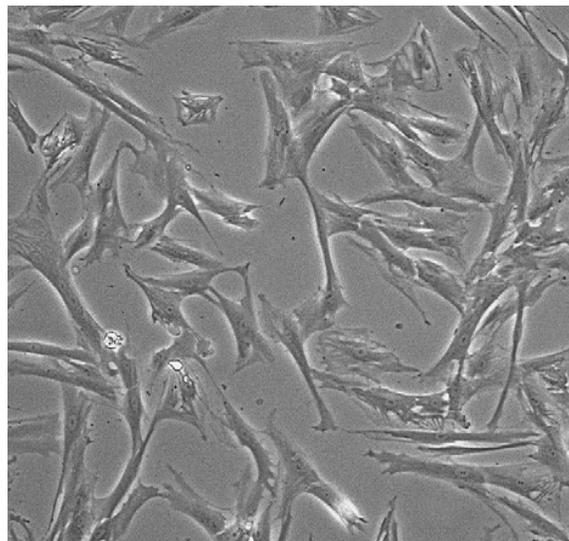
Fig. (3). Typical fibroblast-like morphology of WJ-MSCs expanded *in vitro*.

expansion potential *in vitro* (80 and more population doublings) than adult postnatal MSCs. Second, they are characterized by a much lower expression of HLA-class I, and lack of expression of HLA class-II, surface markers than adult postnatal MSCs, such as bone marrow-derived MSCs [41]. Third, similarly to PD-MSCs and UCB-MSCs, WJ-MSCs possess a broader differentiation potential as adult MSCs. Very recent data shows that WJ-MSCs can be differentiated towards endoderm-derived tissues, such as those of the pancreas and liver [51, 52]. Analogous to umbilical cord blood-derived MSCs, WJ-MSCs also improve properties of osteogenesis and neurogenesis *in vitro* [42]. Unlike postnatal MSCs and PD-MSCs, WJ-MSCs consistently express embryonic stem cell markers like Oct-4, Sox-2, and Nanog. They also express markers of pluripotency, such as SSeA-4 and Tra-1-60. Additionally, WJ-MSCs show much broader characteristics with respect to immunomodulation as postnatal MSCs. For example, it has been shown that WJ-MSCs inhibit T-cell proliferation during mixed lymphocyte assay and tolerated allogenic transplant [12]. Based on these unique properties Wharton's jelly MSCs can be charac-terized as an amenable, plentiful, and inexpensive source of multipotent MSCs with promising potential for use in regenerative medicine applications [48].

APPLICATION OF WJ-MSCS IN CARDIOVASCULAR TISSUE ENGINEERING

Due to the limited regenerative potential of human car-diovascular system, development of functional replacements that support the regeneration of damaged or diseased cardio-vascular tissues, especially for newborn and pediatric patients, is critical. Surgical treatment is commonly based on non-autologous valves or conduits, which have distinct disadvantages including obstructive tissue ingrowths and calcification of the implant [53, 54]. These limitations and the lack of growth typically necessitate re-operations of

pediatric patients with cardiovascular defects, which are consequently associated with an increased risk of morbidity and mortality each time. Therefore, cardiovascular fetal tissue engineering focuses on the *in vitro* fabrication of autologous, living tissue with the potential for regeneration of heart muscle. This promising scientific field aims to address the currently unmet medical need of growing replacements, particularly those for congenital malformation repair [55].

Two adult cell types routinely used for such fabrication of cardiovascular tissues are (I) cells with the capacity to form an extracellular matrix, commonly myofibroblasts, and (II) endothelial cells with antithrombogenic characteristics. Seeding cells onto three-dimensional scaffolds is sequential – myofibroblasts are seeded first, followed by endothelial cells [56]. Due to their better self-renewal capacity, broader plasticity, and immunomodullatory properties comparable to adult fibroblasts, tissue-engineered constructs based on perinatal stem cells in general (and particularly WJ-MSCs) could be an attractive alternative to classical cardiovascular substitutes. WJ-MSCs would be ideal for tissue-engineered constructs as they are autologous, can be harvested through minimally invasive means, possess excellent growth capacities, and are able to form an optimal neo-matrix with excellent mechanical properties. Furthermore, endothelial cells, necessary for the generation of cardio-vascular substitutes, could be isolated either from umbilical cord vessels or umbilical cord blood [57, 58].

The feasibility of using WJ-MSCs as an alternative, autologous cell source for cardiovascular tissue engineering as well as their feasibility in developing pulmonary artery conduits was investigated starting from 2002 [59-61]. Scientists concluded that the *in vitro* fabrication of tissue-engineered human pulmonary conduits was feasible utilizing human WJ-MSCs within a biomimetic culture environment. Interestingly, the morphologic and mechanical features closely approximated those of a native human pulmonary artery. Human WJ-MSCs demonstrated excellent growth properties representing a new, readily available cell source for tissue engineering without sacrificing intact vascular donor structures.

In 2004, researchers studied various umbilical tissues as potential sources for tissue engineering [62]. The study, performed on adult humans, evaluated cells isolated from the umbilical cord artery (UCA), umbilical cord vein (UCV), whole umbilical cord (UCC) and saphenous vein segments (VC) as alternative autologous cell sources for cardiovascular applications. Cells from the UCA, UCV and UCC demonstrated excellent cell growth properties comparable to VC. Following isolation, all three cell groups showed myofibroblast-like morphology and characteristics by staining positive for alpha-smooth muscle actin (ASMA) and vimentin. Histology and immunohistochemistry of seeded polymers showed good tissue and extracellular matrix formation containing collagen I, III, and elastin. Transmission electron microscopy showed viable myofibroblasts and the deposition of collagen fibrils and progressively-growing tissue formation, with a confluent surface, was observed via scanning electron microscopy. UCA, UCV, UCC and VC tissue-engineered constructs did not differ in their mechanical properties. Cell growth, morphology, characteristics and tissue formation were comparable between UCA, UCV, UCC and VC leading to the conclusion that the tissue engineering of cardiovascular constructs by using cells from the UCA, UCV, and UCC is feasible in an *in vitro* environment.

In 2005, the first results related to fabrication of living patches engineered from WJ-MSCs and endothelial progenitor cells (EPCs) were published [63]. Scientists observed seeded patches representing layered, viable, tissue-like structures. The WJ-MSCs in the newly formed tissues expressed myofibroblast markers, such as desmin and ASMA. The EPCs derived neo-endothelia showed constant endothelial phenotypes (CD 31, vWF). Major constituents of ECM such as collagen and proteoglycans were biochemically detected and stress-strain properties of the patches showed features of native-analogous tissues.

One year later, in 2006, the preliminary results were reported for living autologous heart valves based on WJ-MSCs [64, 65]. In this study, biologically-active heart valve leaflets were engineered using prenatally available human umbilical cord-derived progenitor cells as the only cell source. WJ-MSCs and umbilical cord blood-derived EPCs were subsequently seeded on biodegradable scaffolds and cultured in a biomimetic system under biochemical or mechanical stimulation or both. Depending on the stimulation, the leaflets showed mature, layered tissue formation with functional endothelia and extracellular matrix production comparable with that of native tissues. This demonstrates the feasibility of heart valve leaflet fabrication from prenatal umbilical cord-derived progenitor cells as a further step in overcoming the lack of living autologous replacements with growth and regeneration potential for the repair of congenital malformation [55].

The general concept of WJ-MSC-based cardiovascular tissue engineering has also been validated in large animal studies [66]. Precisely, completely autologous, living tri-leaflet heart valves generated using human WJ-MSCs seeded on biodegradable matrices have been successfully implanted in growing sheep models for up to 20 weeks. These valves showed good functional performance as well as structural and biomechanical characteristics strongly resembling those of native semilunar heart valves.

In 2010, the procedure for microencapsulating WJ-MSCs has been reported [67]. This study describes the functional properties in terms of secretive profiles of both free and encapsulated WJ-MSCs. Interestingly, microencapsulation did not alter the morphology and viability of the WJ-MSCs and the encapsulation procedure represents a promising strategy for *in vivo* utilization of WJ-MSCs for possible applications in cardio-vascular tissue engineering and biomedicine. Immuno-protective capsules or devices will be used in regenerative medicine as vehicles for the delivery of different therapeutic agents as well as cells to the injury cite. Because of their specific structural characteristics, such as spherical configuration and small size, microcapsules have much better surface-to-volume ratios than macro-vehicles. Second, microcapsules allow precise tailoring of permeability to allow diffusion of anabolic compounds (oxygen, glucose, etc.) and of cell-derived products (carbon dioxide, lactate, hormones, etc.) while, simultaneously excluding immuno-globulins. Third, microcapsules minimize the overall risk of immuno-

protection failure by using thousands of them instead of a single large macro-capsule. Fourth, they can be injected directly or transplanted with minimal-invasive surgery into the different tissue and organs [68].

Very recently, in 2011 development of myocardial patches based on WJ-MSCs incorporated in 3D aligned microfibers desired for potential treatment of myocardial infarctions and improvement of long term cardiac tissue functions has been described [69]. The experimental 3D construct design is based on two biodegradable macro-porous tubes, which allow transport of growth media to the cells within the construct itself, and cell seeded, aligned fibre mats wrapped around them. The 3D constructs were cultured in a micro-bioreactor with perfusion the growth media transiently through the macro-porous tubing for 14 days. Experimental data confirm that 3D constructs from static and perfused cultures enhanced cell viability, uniform cell distribution and alignment due to nutrient provision from inside the 3D structure.

ADVANTAGES AND LIMITATIONS FOR THE USE OF WJ-MSCS IN TISSUE ENGINEERING

Results from pre-clinical and clinical trials with extra-embryonic WJ-MSCs are still limited recent progress in basic and clinical research of these cells revealed multiple possibilities for their potential applications in regenerative medicine [15, 70]. As mentioned earlier, use of the umbilical cord allows for a rapid initial isolation of large numbers of multipotent WJ-MSCs, with an average amount of 400,000 cells per umbilical cord, avoiding the need for extensive multiplication and potential epigenetic damage [42]. Such amounts of perinatal MSCs are much bigger than the average amounts of postnatal MSCs normally obtained from human bone marrow [71, 72]. WJ-MSCs can also be successfully expanded for long-term use and differentiated *in vitro*. Furthermore, due to their close ontogenic relationship to embryonic stem cells, WJ-MSCs possess unique immuno-modullatory characteristics. All these advantageous characteristics make WJ-MSCs very interesting and promising candidates for different fields of regenerative medicine (Fig. **4**).
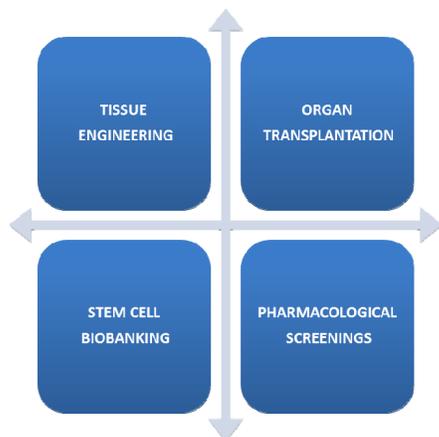
Fig. (4). Potential clinical directions for the use of WJ-MSCs.

Scaffold-based tissue engineering using WJ-MSCs could facilitate the construction of living autologous replacement structures such as vascular grafts and heart valves [73, 74].

In order to fabricate such cardio-vascular constructs, cells would be seeded onto biodegradable scaffolds that are implanted into a biomimetic system "bioreactor", where tissue formation and maturation would accelerate. After a defined time period, sufficient tissue formation have taken place and the constructs were ready for implantation [75]. Scaffold-free tissue engineering based on WJ-MSCs could facilitate stem cell based structures, such as stem cell sheets or micro-tissues, with great potential for the wound healing [76, 77]. Due to WJ-MSCs' unique immuno-modulatory characteristics, such scaffold-free structures even could have potential for use in allogenic transplantations. Currently, the first attempts to use perinatal placenta-derived stem cells (PD-MSCs) for the manufacturing of live, differentiable cell sheets have been successfully demonstrated [78, 79].

The history of biobanking started from 1990th when first banks for storage of UCB were established. Because of a strong biomedical need, stem cell biobanking has led to a growth in stem cell applications [80]. However, umbilical cord blood, containing both hematopoietic and mesenchymal stem cells, is still a predominant source of extra-embryonic MSCs useful for cell biobanking first attempts to store samples of umbilical cords itself have been currently performed. Comparison studies of different MSC sources have shown that WJ-MSCs have significant potential for cell biobanking [81]. In the concept of umbilical cord biobanking samples containing WJ-MSCs could be taken from the placenta post-delivery, frozen, and stored for the long term. On demand, such samples can be thawed, and isolated MSCs can be transplanted into the patient.

Though WJ-MSCs have potential in *in vivo* applications, they can be immediately utilised in pharmacological screening. Presently, many efforts will be performed for development of different cell-based systems as test-objects for determining various drug-related or physiologically mediated effects. Until recently, attempts to use human MSCs for cell-based assays have been hampered by the lack of appropriate transfection methods. Employing of new transfecting systems allow transfection of MSCs with high efficiency without affecting of their functionality. Systems based on single transfected WJ-MSCs, as well as its assemblies, could be utilised to determine different pharmacological, toxicological or pathological effects in perinatal medicine, or instance.

Additional specific field for applications of WJ-MSCs is transplantation of solid organs. It has been demonstrated, that upon systematic administration *ex vivo* expanded MSCs preferentially homed into damaged tissues and participate in regeneration processes through production of specific paracrine factors [12]. As was mentioned above MSCs are able to modulate / suppress immunologic response through interactions with different immune cells. Currently performed investigations indicate that MSCs contribute in inhabitation of rejection and prevention / controlling of GVHD after HSC transplantation. The first report about the potential of bone marrow MSCs for treatment GVHD in 9-year old boy who received a matched unrelated donor HSC transplant has been published in 2004 [82]. As was mentioned above WJ-MSCs as well as other perinatal extra-embryonic MSCs possess advantageous immuno-suppressive

potential, and those can be seen as promising candidates for immunomodulation and treatment of GVHD.

Despite knowledge of its advanced characteristics and first reports of successful pre-clinical and clinical applications, WJ-MSCs require further study to determine its clinical limitations and establish realistic clinical protocols. Firstly, it is unknown if WJ-MSCs engraft in the long-term and display self-renewal and multipotency *in vivo*. Secondly, the amount of WJ-MSCs required for successful clinical applications is huge therefore the problem of scale-up is very important for their successful use in tissue regeneration. For this reason, establishing parameters for (I) GMP-related expansion of sufficient cell amounts and (II) routine clinical applications of GMP-expanded cells are two critical aspects to consider for the prospective transplantation of WJ-MSCs. First attempts to establish critical parameters for generation of clinically-sufficient amounts of WJ-MSCs have been performed only recently [83-85]. Parameters for applications of stem cells on human patients are summarized in the polices of the world marrow donor association (WMDA) as well as in the international standards for cellular therapy, product collection, processing and administration. Currently they are defined for hematopoietic progenitor cells (HPC), human cells, tissues or cellular or tissue-based products (HCT/Ps), and cord blood units (CBU). These parameters include infection disease markers (such as hepatitis B and C, human HIV, HTLV, CMV, syphilis), number of pregnancies, and number of transfusions, for instance. Moreover, successful transplantation is strongly related to the histocompatibility of donor and recipient. Therefore, analysis of donor histocompatibility (specifically for HLA-A, B, C, DRB1, DRB3, and DQB1) is the most important criterion in bone marrow and PBSC transplantation. Unlike HSCs and HCT/Ps, parameters for routine clinical applications of human adult and extra-embryonic MSCs are not yet established. Currently, only the minimal criteria for defining multipotent mesenchymal stromal cells, mentioned above, were summarized as a position statement from the International Society for Cellular Therapy (ISCT) [49]. Thirdly, replacements currently applicable in tissue scaffold-based tissue engineering are mostly based on foreign materials, such as natural, synthetic or hybrid polymers, which result in a lack of growth and remodelling and carry the risks for thrombo-embolic complications and infections. Possible problems concerning these systems are systemic toxicity, growth limitation, differentiation and function restraints, incorporation barriers and cell or tissue delivery difficulties. Thus, the development of compatible biomaterials that do not mitigate WJ-MSC regenerative- and immuno-modulatory-potential is necessary.

CONCLUSIONS

Stem cell based regenerative medicine can be seen as a novel revolution approach for treatment of reconstruction of damaged human tissues and organs as well as broad range of diseases, such as congenital abnormalities and inherent pathologies. Therefore identification and characterization of novel stem cell sources is critical for clinical success of regenerative medicine. Emerging evidence suggests that different compartments of the human placenta are versatile sources of mesenchymal stem / stromal cells, called extra-

embryonic MSCs. These extra-embryonic MSCs, derived from Wharton's jelly, are assigned an intermediate position between pluripotent embryonic stem cells (ESCs) and multipotent adult MSCs, and share specific properties both of these cell types. Due to their close ontogenetic relationship with ESCs, extra-embryonic WJ-MSCs possess extended plasticity, self-renewal capacity, and long-term expansion potential as well as immunoprivileged characteristics comparable with post-natal MSCs. The isolation of extra-embryonic WJ-MSCs is non-invasive and poses no potential risk for the patient. In addition, extra-embryonic tissues represent so called "waste material", which is normally discarded after birth; their isolation is not associated with any current ethical concerns. Experimental results of last decade have shown that WJ-MSCs have great potential in tissue engineering, in which one of the most promising directions for their use is cardio-vascular tissue engineering. Nonetheless, long term survival of the stem cells, in the host tissue after transplantation and establishment of treatment regimen are critical issues which still hampering broad clinical applications of WJ-MSCs. For this reason the establishment of clinically relevant criteria for isolation, characterization, long-term cultivation, and maintenance of human MSCs is absolutely necessary for the successful use of WJ-MSCs in regenerative medicine applications.

CONFLICT OF INTEREST

None declared.

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