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Review

Birth Tissue-Derived Mesenchymal Stromal/Stem Cell Isolation and Assessment

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Abstract

Mesenchymal Stromal/Stem Cells (MSCs) were originally discovered in the 1970s. MSCs are considered a multipotent population that retain tri-lineage differentiation. These cells can be sourced from a variety of tissues, including bone marrow, adipose, molar pulp, and birth tissues. Historically varying definitions of MSCs have existed, but in 2006, the International Society for Cellular Therapy (ISCT) set forth a standardized, minimal criteria for MSCs, including plastic adherence, differentiation into osteoblasts, adipocytes, and chondrocytes, as well as the presence of specific cell surface markers (CD73, CD90, CD105), the absence of hematopoietic surface markers (CD34, CD45, CD11b, to name a few) and a lack or low

expression of MHC Class II markers assessed via flow cytometry. In the current chapter, cell isolation and culturing methods from two differing birth tissue sources of MSCs are discussed. Cell isolation is described using tissue excision and migration as well as enzymatic digestion and plating. Histological and cellular identification markers were used along with flow cytometry applications, as outlined by the ISCT. Proliferation potential and growth kinetics are evaluated, and a description of trilineage differentiation has been provided. Lastly, a brief outline of the regenerative potential using MSCs in clinical applications is discussed.

Keywords
Mesenchymal stem cells; stem cell; cell isolation; birth tissues

1. Introduction

The early identification of mesenchymal stromal/stem cells (MSCs) started when Friedenstein and colleagues in the 1970s identified a population of non-hematopoietic fibroblast-like, spindle-shaped cells isolated from the stroma of bone marrow and spleen, which retained the ability to differentiate into adipocytes and osteoblasts [1, 2]. In 1991, Caplan identified these fibroblast-like cells in adult bone marrow and periosteum as MSCs and determined they could differentiate into osteoblasts and chondrocytes [3]. Additional research further identified that these adult tissue-derived MSCs could differentiate into connective tissue types of mesenchymal origin [4]. Cells with similar stem-like properties were identified in placental tissues: amniotic epithelial cells, amniotic MSCs, chorionic MSCs as well as MSCs from chorionic villi, umbilical cord, Wharton's jelly, and the maternal decidua [4-14].

While there is no single biomarker or characteristic that will positively identify human MSCs isolated from an array of tissue sources [15], a set of minimal criteria were established by the International Society for Cellular Therapy (ISCT) in 2006 to define human MSCs. The MSC must exhibit plastic adherence, express CD105, CD90, CD73, and lack expression of hematopoietic cell surface markers CD34, CD45, CD14, CD11b, CD79α, CD19, as well as a lack of expression of HLA-DR markers. Finally, these cells must be able to differentiate into osteoblasts, adipocytes, and chondrocytes *in vitro* [16]. This multilineage differentiation potential of MSCs is an attractive aspect that has expanded their suitability for a variety of therapeutic applications [17]. In addition to their trilineage differentiation, MSCs are known for their self-renewal capability, immunomodulatory properties, and regenerative capabilities [18].

There are multiple sources of adult MSCs, for example, all organs contain perivascular MSCs [19]; however, sizeable quantities, ranging in the several millions, of adult MSCs are most easily collected from bone marrow and adipose tissue by standardized medical procedures, and are generally isolated for autologous treatment. There is a plethora of literature on the clinical uses of MSCs from both Bone Marrow (BMMSC) and Adipose (ASC) for autologous therapy in the United States [20-23]. Although these cells are used in a clinical setting, they do demonstrate decreased fitness with age. Stolzing et al., demonstrated BMMSCs decline in fitness with increasing age. BMMSCs from young (7-18 years), adult (19-40 years), and aged (>40 years) donors exhibited

reduced proliferation and differentiation potential, increased reactive oxygen and nitrogen species, correlating with decreased superoxide dismutase levels and diminished heat shock protein stress responses [24]. Studies have been performed on ASCs with similar results [25, 26] In contrast, MSCs isolated from birth tissue can be collected in large numbers and have demonstrated the ability to retain their proliferation and differentiation potential through several population doublings [27].

Birth tissue-derived, placental MSCs are innately immune-privileged due to their low to moderate expression of MHC Class I antigens, general absence of HLA-DR antigens, and the presence of HLA-G, which confers immune protection [9, 28-30]. They also exhibit immunomodulatory properties, suppressing several functions of immunocompetent cells and contributing to anti-inflammatory responses with the increased production of IL-10 and TGF-β [6, 28-33]. Due to these anti-inflammatory responses, MSCs are continually being evaluated for use in systemic treatments of autoimmune diseases, such as sclerosis and rheumatoid arthritis [34-37]. Birth tissue MSCs have been successfully isolated from whole placenta and embryonic tissues, such as amnion, chorion, umbilical cord, umbilical cord blood, and Wharton's jelly [5-13, 31, 38-42]. MSCs derived from birth tissues maintain their inherent regeneration potential and demonstrate high differentiation and proliferative capacities [43, 44]. However, unlike other sources of birth tissue MSCs, MSCs isolated from whole placenta, including the *decidua parietalis*, will include maternal-derived MSC populations expressing maternal HLA-DR antigens that will elicit an immune response *in vivo*.

Many approaches to disease modelling have previously revealed that MSCs hasten wound recovery and suppress tumor development. Clinical interest in MSCs derived from Wharton's jelly within umbilical cord tissues has been an active area of research due to their promising immunoregulatory properties when used as an allogeneic cell source for transplant purposes [42, 45-48]. Uniquely, umbilical cord MSCs (UCMSC) have been shown to express embryonic transcription factors (Oct-4, Sox-2, and NANOG) that play a regulatory role in self-renewal and pluripotency [49, 50]. As such, the potential of UCMSCs for direct or indirect clinical applications avoid the ethical concerns of embryonic cells while retaining a significant potential for differentiation and self-renewal. UCMSCs have also been shown to be safe for transplant without teratoma formation [51, 52].

Despite these potential advantages, clinical trials using MSCs have found limited success. Investigations have shown poor MSC 'homing' (site specific engraftment) to be the main limitation to successful treatments [53, 54]. Additionally, the pluripotency profiles of UCMSC seem to be inconsistent across studies [55, 56]. Typically, when MSCs are isolated to amplify a pluripotent population, they are cultured without additional specific growth factors (GF) or any extracellular matrix (ECM) components in a basal growth medium (BGM) with high glucose and serum concentrations; significantly different than conditions during pregnancy. Previous *in vitro* investigations have demonstrated that MSCs cultured in a BGM supplemented with discrete growth factors have increased cellular growth rates, reduced cell death, and increased regenerative properties [57].

In summary, amniotic, chorionic, and umbilical (Wharton's jelly) MSCs are desirable because they can be readily isolated in high quantities and purity without maternal cell or erythrocyte contamination and are relatively immune privileged [28, 39]. In the current chapter, methods used

to process birth tissue to yield MSCs for *in vitro* culture, growth media conditioning and potential therapeutic application are discussed.

2. Materials and Methods

2.1 Amniotic and Chorionic Mesenchymal Stromal Cells (AMSC), (CMSC)

The amnion is a thin, membranous sac that surrounds the fetus in utero. It is comprised of a thin, avascular membrane, with an epithelial monolayer of squamous or cuboidal epithelial cells that are in contact with amniotic fluid *in vivo*. This tissue contains a thick basement membrane, and avascular stroma, Figure 1 [58-61]. Amniotic MSCs are found in the stromal layer, which is attached to the chorion, a vascularized membrane [5, 6, 39, 60]. To harvest the MSCs from the amniotic membrane, the membrane must first be mechanically separated from the chorion and the rest of the placental tissue, Figure 2 [58]. This separation is accomplished by simple mechanical traction, Figure 2 [38, 39, 58, 59]. MSCs can also be isolated from other birth tissues, such as chorion [8, 38, 40, 41, 62], umbilical cord [41, 62, 63], umbilical cord blood [6, 64], Wharton's jelly [12, 13, 31, 65-67], and the whole placenta [40, 68], with unique procedures for each tissue.

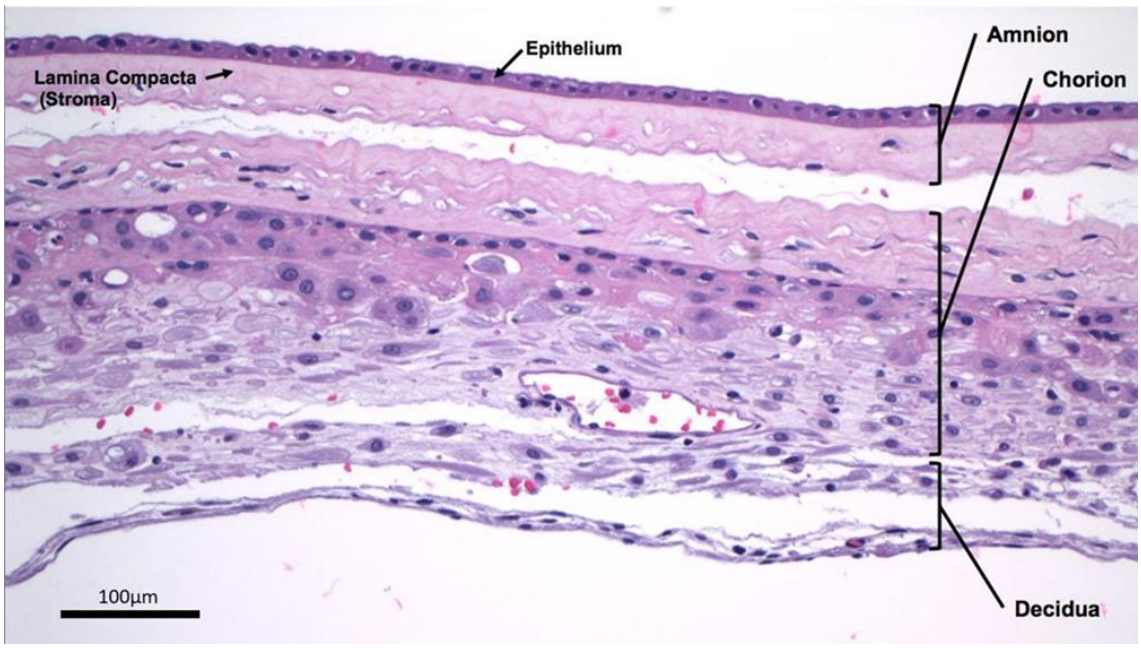

Figure 1 Photomicrograph of native placental membranes. Hematoxylin and Eosin stained cross-section of placental membranes. Image taken on a camera coupled to an Olympus BX43 microscope, 20X magnification, Scale bar = 100µm.

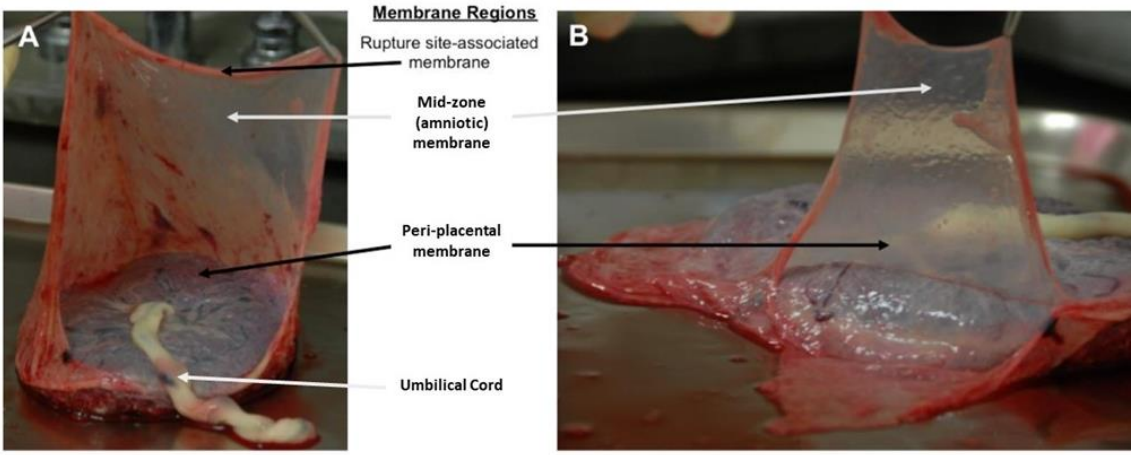

Figure 2 Placental tissue procurement and dissection of amnion from the mid-zone region. (A) Demonstrates the amnion still attached to the chorion and placenta in the orientation similar to *in situ*. (B) The amnion is peeled from the chorion and shows the peri-placental and part of the mid-zone amniotic membrane.

The amnion is physically evaluated and washed to remove any blood associated with chorionic villi that remain, using a saline solution, generally including a combination of antimicrobial and antifungal agents, such as penicillin-streptomycin (P/S), and amphotericin B [60]. Manual washing may not remove all the visible blood from the villi. Post-wash processing is important to ensure that all visible villi are manually removed [60]. Some protocols recommend at this stage to cut the membrane into several pieces, between 4 cm^2 to 100 cm^2, before further processing [9, 10, 61, 69-71]. Magatti et al. also recommends a sterilization step, where the membrane fragments are momentarily exposed to a 0.25% povidone iodine solution before incubation in a beaker containing phosphate-buffered saline (PBS), P/S, amphotericin B, and Cefamezin [60].

The cells of interest, the AMSCs associated with the stromal layer of the amniotic membrane, can then be isolated through enzymatic digestion [8, 60, 61, 68-70, 72, 73]. Some protocols recommend the use of a several step process with a combination of enzymes, such as dispase [8, 60, 72], different collagenases [44, 60, 69, 71, 73] and DNase [8, 60, 73]. Others recommend using trypsin, or trypsin-like enzymes such as TrypLE, along with EDTA [60, 61, 69, 70, 73]. Several protocols call for two-stage protocols, using trypsin to isolate human amniotic epithelial cells (AECs) from the epithelial portion of the membrane first, followed by AMSC isolation by collagenase, dispase or papain and DNase [9, 39, 74]. Barbati et al., describes an approach to isolate high purity populations of MSCs and AECs by first scraping the stromal layer with a glass slide to remove the MSCs, then digesting the AECs from the scraped membrane [39]. The concentrations, selections of digestive enzymes, length of incubation, and temperature of incubation (whether room temperature or 37°C) vary, but are generally followed by a filtration step where the supernatant is passed through a filter between 70 µm to 100 µm to remove large cell clumps and pieces of tissue, followed by centrifugation at speeds varying from 150 x *g* to 500 x *g* [8, 60, 61, 68, 72, 73]. The supernatant is aspirated, and the cells are resuspended in media, then evaluated using Trypan blue exclusion and counted with a hemocytometer to determine cellular number and viability [39, 60]. Viable MSCs exclude trypan blue and appear clear, perfectly round cells, and when adherent have a spindle-like, bipolar fibroblastic morphology, Figure 3D.

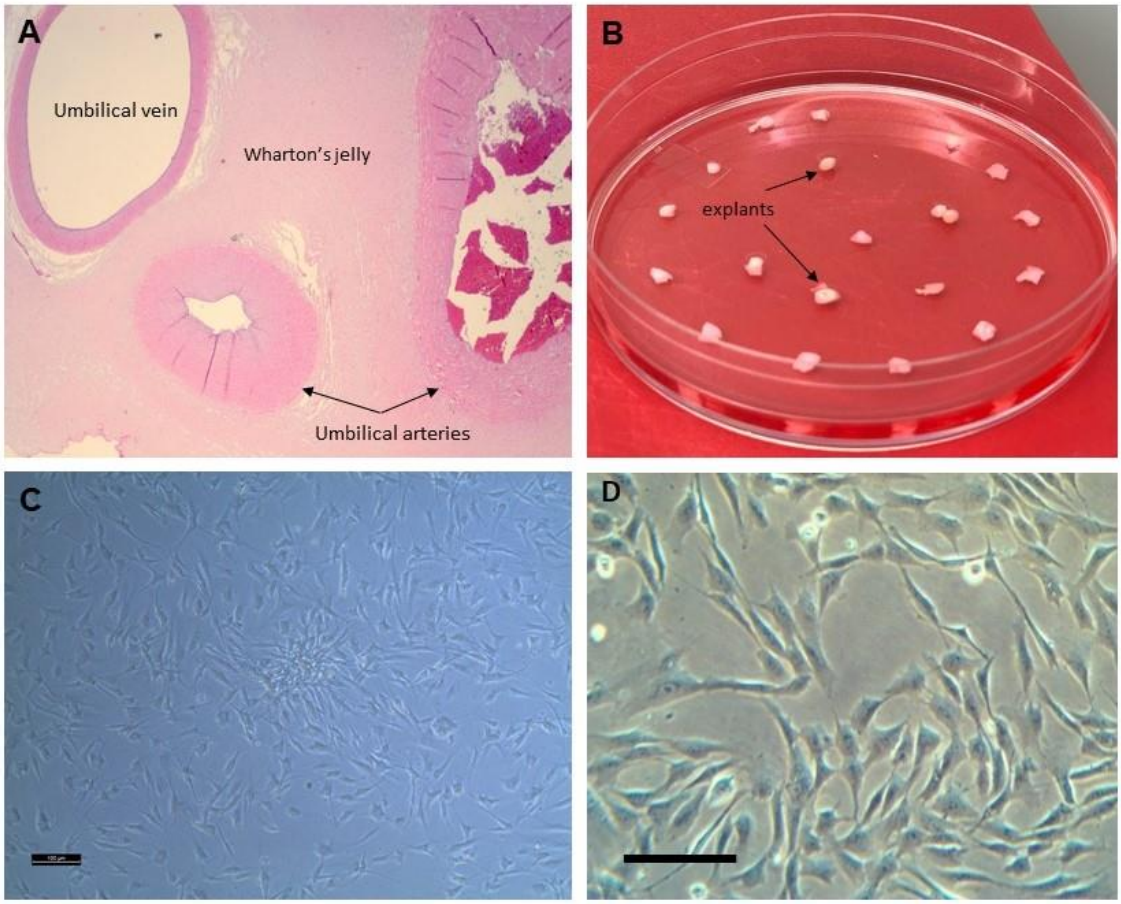

Figure 3 Primary cell isolation from the human umbilical cord. (A) The structure of the human umbilical cord is demonstrated with an H&E stained cross-section. The umbilical cord is composed of two umbilical arteries and one vein supported by the GAG-rich Wharton's jelly that confers resistance to compression. (B) Small sections of tissue are dissected from the Wharton's jelly region of the umbilical cord and cultured *in vitro* to create primary explants of UCMSC. (C) Expanded UCMSCs demonstrating, plastic adherence, bi-polar fibroblast-like structure (20X). Scale bar = 100µm (D) Expanded AMSCs demonstrating, plastic adherence, bi-polar fibroblast-like structure (40X) Scale bars = 100µm.

2.2 Human Umbilical Cord Mesenchymal Stromal Cells (UCMSC)

Human UCMSCs can be isolated from umbilical cords by removing blood vessels, dissecting the Wharton's jelly extracellular matrix into 2-3 mm^2 pieces and plating the explants onto culture plates in suitable media containing penicillin-streptomycin. After 10-14 days of culture, MSCs begin to migrate out of the explants.

2.3 Cell Expansion and Characterization

Cells are seeded into culture flasks and maintained in selected media depending on the application. The type of media used to maintain and expand cells varies, but includes Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% to 20% fetal bovine serum (FBS) [49,

53, 62, 69, 75, 76], Minimum Essential Medium Alpha (α-MEM) [10], Roswell Park Memorial Institute (RPMI) media [5, 8, 72], or other media which facilitate the growth of MSCs in culture [77] or a xeno-free media for therapeutic applications. The initial seeding density and culture area size varies from 4,000 – 50,000 cells/cm^2 [60, 70, 71], and a 25 cm^2 culture flask is common for the initial seeding [62, 70, 71]. The cells are monitored daily, and media is changed every 2-3 days, while confluence is monitored. Once confluence reaches 70-80%, the cells are detached, counted, and further expanded. This process is repeated until the desired number of cells or passages is reached [60, 73].

2.4 Immunophenotypic Characterization

Undifferentiated MSCs can be analyzed by immunohistochemistry for the expression of MSC surface markers (CD105, CD90, CD73, CD45, CD34, CD31) by washing and fixing with 10% neutral buffer formalin, staining with a fluorescent-conjugated antibody and imaged with a fluorescent microscope.

2.5 Flow Cytometry

MSCs are characterized for the presence or absence of specific cell surface markers, established by the ISCT, specifically positive staining for CD73, CD90, CD105, and lack of, or low level, staining for hematopoietic cell surface markers CD34, CD45, CD14, CD11b, CD79α, CD19, and HLA-DR markers (16). Typically, adherent MSCs are detached using dissociation reagents such as trypsin and 0.02% EDTA and resuspended at a concentration of $1x10^7$ cells/mL in appropriate antibody stain buffer and labeled according to kit manufacturer's recommendations.

2.6 Assessment of Pluripotency Gene Expression

Two frequently used methods for assessing pluripotency gene expression include flow cytometry, which requires cellular permeabilization prior to staining [5, 10, 11, 27, 71, 74, 75] and quantitative PCR, normalized to GAPDH, a common housekeeping gene [8, 11, 51]. UCMSCs at passage 3 demonstrate high gene expression levels for CD105, CD90 and CD73, as well as integrin marker (CD29), low expression levels for CD34 and CD31, and high expression of pluripotency marker genes including Oct4, Sox2, and NANOG, Figure 4C.

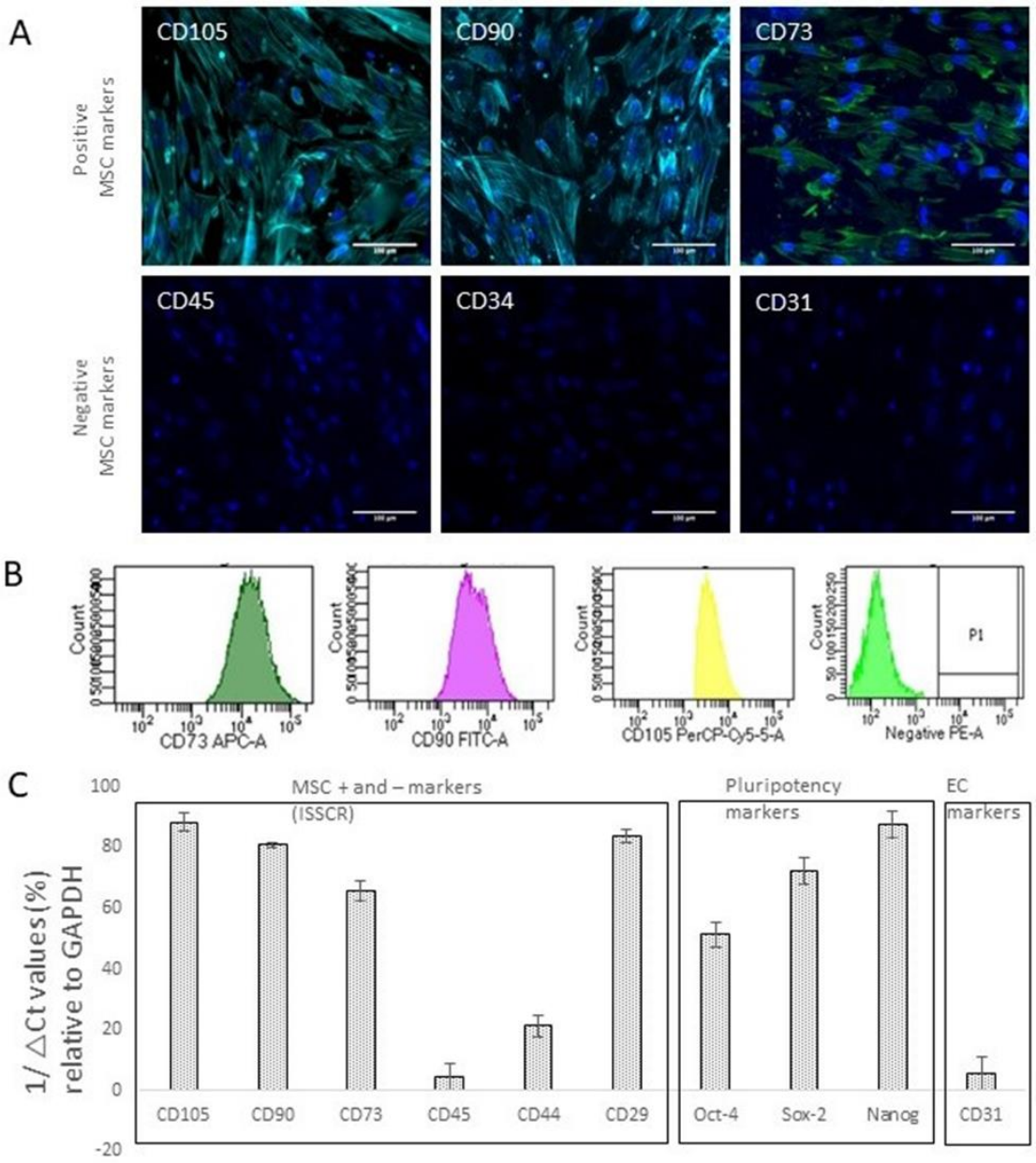

Figure 4 Stemness characterization of UCMSC. (A) The most common positive mesenchymal surface markers (CD105, cyan; CD90, cyan; CD73, green) are analyzed using immunohistochemistry. MSC do not express CD45, CD34 and CD31. Scale bars shown are 100µm (B) Surface marker labeling quantified by using flow cytometry shows high levels of stem cell markers and low levels of CD34, CD11b, CD19, CD45, and HLA-DR (C). Relative mRNA levels of mesenchymal and pluripotency-associated genes are evaluated using RT-PCR with GAPDH as housekeeping control (n=3). USMSCs express high mRNA levels of pluripotency regulatory genes (Oct-4, Sox-2, NANOG).

2.7 Trilineage Differentiation

Multipotent MSCs are also defined by the ability to differentiate into the three mesenchymal lineages, adipocytes, chondrocytes, and osteoblasts [8, 10, 17, 27, 40, 70, 73, 75]. Select media, supplements, and protocols are used to differentiate AMSCs, CMSCs, and UCMSCs into the three

mesenchymal lineages. Literature also supports the differentiation into myogenic, angiogenic, cardiomyogenic, hepatic, pancreatic and neural lineages [6, 9, 10, 27, 28, 70]. Osteoblasts are confirmed by an increase in alkaline phosphatase expression [71] and the presence of calcified structures by Alizarin red or von Kossa staining [9, 10, 27, 42, 65, 70, 74, 78, 79], Figure 5A. Adipocytes are identified by the accumulation of lipid-rich vacuoles, positively stained with oil red-O stain [9, 10, 27, 42, 62, 70, 74], Figure 5B. Chondrocytes are assayed for the presence of proteoglycans by Alcian blue 8GX staining [9, 62, 71, 74] or Safranin O [42], and the presence of collagen II and absence of collagen I and III [9, 27, 62, 70], Figure 5C.

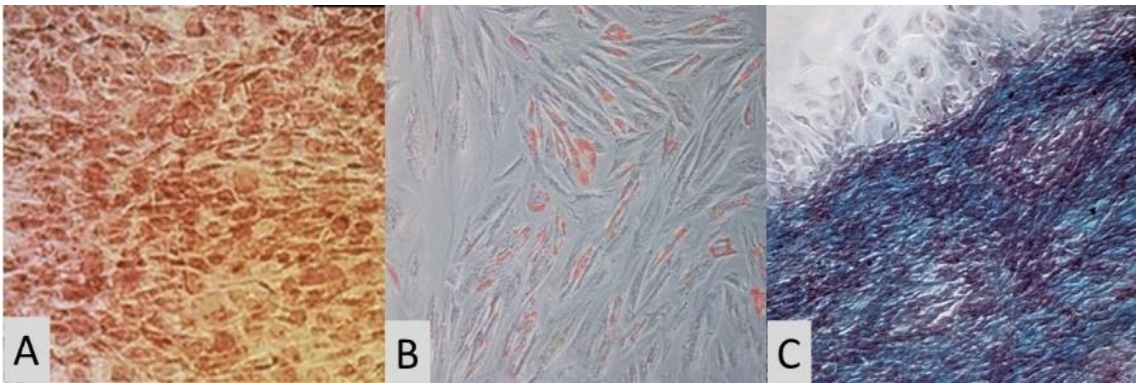

Figure 5 Trilineage MSC differentiation. (A) Alizarin red S staining of human amniotic MSCs following osteogenic induction (40x). (B) Oil Red O staining of human chorionic MSCs following adipogenic induction (40x). (C) Alcian blue staining of human chorionic MSCs following chondrogenic induction (40x). A Used with permission from "Comparable osteogenic capacity of mesenchymal stem or stromal cells derived from human amnion membrane and bone marrow," by M Ghasemzadeh, et al., 2018, Cytotechnology. © 2018, Springer Netherlands. B & C Used with permission from "Isolation and Characterization of Chorionic Mesenchymal Stromal Cells from Human Full-Term Placenta," by BK Koo, et al., 2012, Journal of Korean Medical Science. © 2012, Korean Academy of Medical Sciences.

3. Results

Human AMSCs and UCMSCs were both collected from primary tissues and evaluated for their use as potential clinical therapies. Cells were isolated from the amnion and Wharton's jelly of the umbilical cord using enzymatic release from the tissue, while the UCMSCs were collected as they began to migrate out from small cut pieces of the tissue source. The AMSCs were maintained using a serum and xeno-free media, while the UCMSCs were maintained in a complete growth media containing FBS. Both cell lines demonstrated similar morphological characteristics.

3.1 Stemness Characteristics of Birth Tissue Derived MSC

UCMSC cell surface marker expression, detected by immunohistochemistry were positive for CD90, CD73 and CD105 and negative for CD45 (leukocyte surface marker), CD34 (endothelial progenitor cells marker), and CD31 (endothelial cell marker), Figure 4A. These cells were further validated by flow cytometry with a strong positive expression (>98%) for CD105, CD90 and CD73

and negative expression (<2%) for HLA-DR, CD11b, CD34, CD79a, and CD45, Figure 4B. UCMSCs demonstrated high gene expression levels for CD105, CD90 and CD73, as well as integrin marker (CD29), and lower expression levels for CD34 and CD31 (Figure 4B). The high expression of pluripotency marker genes included Oct4, Sox2, and NANOG were detected from early passaged (P3) UCMSC, Figure 4C.

3.2 Characterization of Proliferation Kinetics

Cells migrated out from small pieces of umbilical cord Wharton's jelly during primary culture. At P0, UCMSC demonstrated smaller and round morphology and after longer culture times, they began to display myofibroblast-like morphology, Figure 3C. After 14 days, MSCs in BGM appeared as more spindle-like cells, and a portion of cells in the ECGM started to form cobblestone-like structures (Figure 6A, 6B). UCMSC displayed higher cumulative population doubling levels during cell passages (P6-20). Doubling level of Wharton's jelly MSCs increased until P16 with a peak of 22.32 in BGM, and that of UCMSC in ECGM with a peak of 17.91 increased until P18, indicating that UCMSC in ECGM had lower proliferation rates than the cells in BGM (Figure 6C).

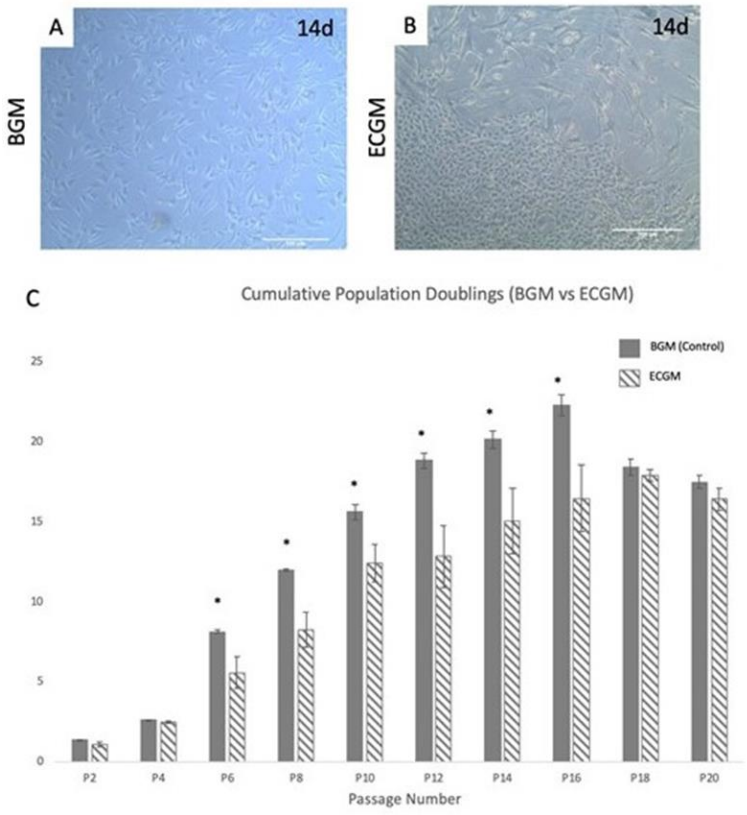

Figure 6 (A) and (B) Morphological analysis and population doubling level of UCMSC cultured in BGM (control) and ECGM. UCMSCs cultured in BGM and ECGM display morphological differences. Scale bars = 100µm (C) The number of UCMSC counted from passage 2 to 20. Cumulative population doublings (CPD) are calculated based on cell counts. In early passage numbers, there is no significant difference between media culture conditions. From P6 to P16, MSC proliferation capacity is higher in BGM than ECGM. (*=$p<0.05$)

4. Discussion

Human MSCs are capable of self-renewal and retain the potential to differentiate into multilineage cell types, including vascular cells and are currently being used for cell-based therapies and tissue engineering applications [78-82]. MSCs can be readily isolated from a range of birth tissues through a variety of methods, producing heterogeneous MSC populations. The advantages to collecting birth tissue-derived MSCs for clinical use include; easy access to source tissue, large quantities of MSCs per tissue, ease of culture, multilineage differentiation potential, and subsequent application in a range of regenerative medicine applications [8, 17, 27, 43, 44, 47, 81, 83]. When considering the type of tissue to use for the collection of MSCs, cell heterogeneity should be considered. Collecting MSCs from the entire placenta results in a higher degree of heterogeneity. As the organ is dissected into smaller constituent tissues the heterogeneity of the cells decreases. MSC collection from the amnion, for example, decreases heterogeneity to include amniotic epithelial cells and AMSCs, and these cells can be isolated separately. In the literature, the majority of birth tissue MSCs are a heterogeneous population as evidenced by the range of cells that are positive for the various MSC specific markers, MSC-specific vimentin, stem markers, such as Oct-4, SSEA-4, Klf4, NANOG, and negative of epithelial cell markers (E-cadherin, cytokeratins). Kobayashi et al. demonstrated a side population of AMSCs, in which the majority were negative for classical MSC markers CD90 and CD105, negative for hematopoietic markers CD34 and CD45, negative for MHC Class I and II antigens and expressed the pluripotent markers Oct-4, SOX2, Nestin, and Rex-1. These MSCs can be expanded *ex vivo* up to 40 population doublings and are able to differentiate into not only the standard mesenchymal lineages (adipocytes, chondrocytes and osteoblasts), but also into the neuroectoderm lineage into neurons [74].

Similarly, dissecting the Wharton's jelly from the umbilical cord versus using sections of the ECM within the cord decreases heterogeneity. Cells from the Wharton's jelly of the umbilical cord show high expression levels of the common MSC markers (CD73, CD90 and CD105) together with relatively high expression of pluripotent stem cell markers (Oct4, Sox-2, and NANOG). These transcription factors are expressed in the cells derived from gelatinous tissue using the explant method in early passages (P3) but not observed in either semi-differentiated cells from umbilical cord ECM or late passage cells (P20) [50, 79, 84, 85]. Moreover, CD45, CD34, and CD31 expression is absent, thus confirming that isolated UCMSCs possess greater populations of primitive MSCs absent of non-stem cell contamination. The phenotypic profiles of UCMSCs isolated from distinct regions appear to be inconsistent in different research studies and requires better identification for successful clinical application. The *in vitro* studies demonstrate that similar to other MSCs, stem cells from umbilical cord display typical fibroblast-like morphology and also originate from the different compartments of the umbilical cord [56, 85].

The process used to collect primary cells should also be considered. To collect larger quantities of cells (several million of Passage 0) from birth-tissues, enzymatic digestion of the tissues is recommended. This will provide the technician with a larger amount of heterogenous cells, which would require further cell selection for use in clinical therapies. Enzymatic digestion also allows plating and seeding cells the same day the digestion occurs. If the dissection of tissue and explant plating method is used, a lower quantity of more homogenous cells is collected (several hundreds

of thousands at passage 0) but will take several weeks to allow cells to migrate and proliferate from the tissue sources.

Specific markers for MSCs can vary with the source of the cells but have also been shown to vary with passage number [10, 60, 86]. When performing flow cytometry to assess immunophenotype of cell cultures, it is recommended to compare populations of cells with their respective passages to ensure the target phenotype is being maintained, as recommended in Magatti et al. [60]. By keeping a cryopreserved batch of each preparation after isolation and passaging, and performing flow cytometry on each passage, a proper basis of comparison for immunophenotyping can be obtained [60]. Currently, there is no consensus on the specific markers for flow cytometry beyond the general guidelines put forth by the ISCT in 2006 [16]. This is mainly due to the variance of cell markers expressed by MSCs depending on source tissue and passage number. Flow cytometry is still one of the most reliable tools available for phenotypic characterization.

For therapeutic application in the clinic, there are several considerations when selecting methods, reagents, and culture media. When choosing reagents and media, if possible, use xeno-free and serum-free reagents devoid of human or animal-derived components [87]. TrypLE Select (ThermoFisher) is an example of a suitable xeno-free trypsin replacement, this reagent is not only xeno-free but is processed on xeno-free equipment. When using trypsin or a trypsin-like enzyme for cellular dissociation, it is important to use calcium and magnesium-free reagents when working with the membrane before enzymatic digestion. Calcium and magnesium promote cell to substrate binding [86] and chelate trypsin [87]. During enzymatic digestion or release of the cells with trypsin, the removal of the enzyme from the final product should be considered. Trypsin is derived from the pancreas of pigs and can cause reactions in persons with porcine allergies. There may also be religious reasons for removing trypsin from the product as well.

Blood removal from the tissues should also be a consideration when washing the membranes; large amounts of blood may cause a reaction if the cells are collected and used in allogeneic intravenous transplantation [15]. Typically, the components of blood will be washed out after several washes and enzymatic release. Another consideration in creating a clinical therapy is the use of dimethyl sulfoxide (DMSO) in cryopreserved cell stocks. DMSO, a chemical compound used in many therapies and treatments that are FDA approved, is a common cryoprotectant. Frozen cells thawed for cell-based therapies, using DMSO, often require a wash-out step or dilution before *in vivo* application due to its cytotoxicity. DMSO has also demonstrated adverse reactions, such as stroke and myocardial infarction, when used intravenously for systemic therapy [88-90]. A consideration should be to select a DMSO-free cryoprotectant [91, 92].

Birth tissue MSCs are innately immune-privileged, making them an attractive option for treating different diseases. Their low level, or lack, of surface MHC Class Type II antigens protects them from host immunological defences and their immunomodulatory properties down-regulate the host immune response, contributing to anti-inflammatory responses. [9, 29, 30, 36]. Human MSCs from adult (e.g. bone marrow, adipose) or placental tissues (e.g. amnion, chorion, umbilical cord) sources have similar immune privilege and immunosuppressive capacities; however, there is conflicting evidence about whether adult or placental-derived MSCs retain their immune privilege upon differentiation [63, 65, 93-95]. Additional research is needed to fully understand the immunogenic fate of undifferentiated and differentiated adult and birth tissue MSCs within the recipient.

The combination of immunomodulatory properties, the ease at which they can be cultured, and their regenerative potential make birth tissue-derived MSCs a broadly applicable treatment source with a wide variety of clinical applications including personalized medicine, degenerative diseases, and immune system-related diseases [95]. Clinical studies exploring the regenerative potential of birth tissue MSCs have been completed or are underway as of May, 2020 addressing a wide array of conditions including idiopathic pulmonary fibrosis [96], Type I and Type II diabetes summarized by Moreira [97], systemic lupus erythematosus [62], knee osteoarthritis (clinicaltrials.gov NCT 03337243), preterm infant bronchopulmonary dysplasia [98], and a 2015 summary of the uses of placental derivatives by Silini et al. [99]. Research related to birth tissue-derived MSCs continues, expanding our understanding of the regenerative, immunomodulatory and therapeutic applications that can positively impact human diseases.

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Author Contributions

All listed authors contributed to this book chapter.

Competing Interests

The authors have the following relevant disclosures:

Robert B. Diller is employed by Amnio Technology; Robert G. Audet is employed by Axolotl Biologix; Eric T. Lee is employed by Axolotl Biologix; Aaron J. Tabor is employed by Axolotl Biologix; Peter McFetridge is employed by 42 Bio.; Robert S. Kellar consults for Axolotl Biologix.

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