R E V I E W

# An Overview on Mesenchymal Stem Cells Derived from Extraembryonic Tissues: Supplement Sources and Isolation Methods

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**Purpose:** The main aim of this review was to provide an updated comprehensive report regarding isolation methods of MSCs from human extra embryonic tissues, including cord blood, amniotic fluid, and different parts of the placenta and umbilical cord, with respect to the efficacy of these methods.

**Results:** Extra embryonic tissues are the most available source for harvesting of mesenchymal stem cells (MSCs). They make a large number of cells accessible using non-invasive methods of isolation and the least immune-rejection reactions. A successful culture of primary cells requires obtaining a maximum yield of functional and viable cells from the tissues. In addition, there are many reports associated with their differentiation into various kinds of cells, and there are some clinical trials regarding their utilization for patients.

**Conclusion:** Currently, cord blood-MSCs have been tested for cartilage and lung diseases. Umbilical cord-MSCs were tested for liver and neural disorders. However, these MSCs can be isolated, expanded, and cryopreserved in a cell bank for patients in need.

**Keywords:** cord blood-MSCs, umbilical cord-MSCs, amniotic fluid, placenta, embryonic tissues, immune-rejection

## Introduction

Mesenchymal stem cells as connective tissue cells are located in the extracellular matrix (ECM);[1] the non-living viscous substance in which cells and fibers belong to the ECM. ECM is a collection of glycoproteins, collagens, laminins, fibronectin, elastin, and proteoglycans, chondroitin, heparan, keratan sulfates, and hyaluronic acid.[2] Mesenchymal stem cells are an important cell population, which can possess stem cell-like characteristics.[3,4] They are multi-potent, and found in nearly all tissues, mostly located in perivascular niches (Figure 1).[5] Perivascular location of the mesenchymal stem cells (MSCs) correlates them to pericytes.[6] Pericytes exist in the wall of the blood vessels, and closely encircle endothelial cells in capillaries, and micro vessels in multiple organs.[7] According to this, MSCs can stabilize blood vessels, and contribute to tissue, and immune system homeostasis under physiological conditions. They play a more active role in the repair of damage of the local tissue.[8] Since the MSCs have migratory abilities, they secrete protective factors and act as a primary matrix for tissue regeneration during inflammation, tissue injuries, and certain cancers.[5] MSCs can modulate the function of different cells of the immune system, eg, T cells, B cells, natural killer cells, and dendritic cells. The in vitro immunomodulatory properties of

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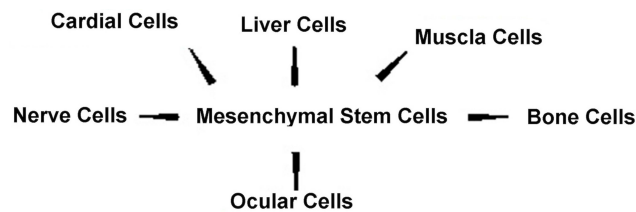Fax +9834331325215
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**Figure 1** Schematic illustration of mesenchymal stem cells extracted from umbilical cord blood as source multi-potent and found in nearly all tissues.

MSCs cause more interest on the potential applications of them in in vivo assay (as an immunosuppressive cellular therapy).[9] MSCs exhibit a broad differentiation capacity to osteoblasts, adipocytes, and chondrocytes, hepatocyte-like cells, neuronal, and neuroglial cells.[10] They expressed mesenchymal markers such as CD105 and CD90 positively.[11] Also MSCs are isolated from a variety of adult tissues, eg, peripheral blood tissues, adipose tissues, compact bone, dental pulp, and bone marrow, dermis, pancreatic islets, adult brain tissues, skeletal muscle tissues, and synovium, circulatory system tissues,[7] lung, heart, and hair follicles.[8] Foetal tissues such as liver, lung, and neonatal tissues including placenta, amnion and different parts of the umbilical cord,[12] and cord blood[5] are used to isolate the MSCs. As an alternative, stem cell populations could be harvested at birth.[10] In this review, the isolation methods of MSCs from different supplement sources were discussed, and also their utilizations in medical application were explained.

## Cell Isolation Protocols

Dissociation of the animal cells depends on the age, species, tissue origin, and the type of dissociation medium. Tissue and species factors are not controllable, but mediums and conditions associated to tissue dissociation could be controlled to get the best results.[13] Generally, two methods, eg, enzymatic and non-enzymatic are used for cell isolation. Enzymes are chosen according to the connective tissue matrix of the particular organ.[14] Enzymes show different impacts on the morphological characteristics of the isolated cells.[12] They are applied in simple or cocktail form.[15,16] Trypsin and collagenase are common enzymes, which are used in cell separation. Furthermore, some characteristics, eg, kinds of enzymes, amount of impurities in any stock preparation, enzymes' concentration, and temperature, have a successful influence.[13,15,16]

Non-enzymatic methods showed advantages over enzymatic methods, eg, preventing damage to the cell membrane,

and intracellular structures. They are divided into mechanical and chemical methods. Mechanical methods include homogenizing, sieving, mincing, and affinity. Some of them are traumatic for tissue. This showed that the combination of two methods could be more effective if the mechanical methods are applied immediately before placing into the enzyme. Chemical methods are mostly mild, but they have not been used for the isolation of the cells on a large scale. Chemical methods interact via destroying cell-to-cell attachment by removal of calcium, and magnesium, separating cell layers by affecting glycine, and changing the pH through suspension in a buffer solution. Growth factors, eg, epidermal growth factor (EGF), Vascular endothelial growth factor (VEGF), or fibroblast growth factors (FGF), have been used as supplements for the in vitro expansion of isolated MSCs.[17]

## Isolation Methods of Mesenchymal Stem Cells

### Human Umbilical Cord Blood

Umbilical cord blood (UCB) is indicated as a source of hematopoietic stem cells which are transplantable for clinical practice for a decade.[18] Because of the small amount of MSCs, freshly donated UCB does not qualify for transplantation.[19] The isolated amounts of MSCs are affected by the volume of a UCB sample as a criterion.[20] The volume of the UCB unit and the mononuclear cell count are predictive of the isolation rate of MSCs. The mean volumes of the UCB unit and number of nucleated cells per unit are 40 mL and $5.39 \times 10^8$, respectively. But, MSCs can successfully be isolated from UCB units with a volume of $\geq 54$ mL containing $\geq 1.28 \times 10^8$ mononuclear cells, which results in a MSC isolation rate of >70%.[19] In addition, the ratio of cord blood volume/number of nucleated cells is greater for boy neonates than for girls: 78 mL/$12.4 \times 10^6$ cells from boys compared with the 66.6 mL/$10.2 \times 10^6$ harvested cells from the girls.[21] Another parameter for successful isolation of the cells is a transporting time of less than 15 hours from collection to isolation. However, some researchers believe that the MSC isolation rate is not affected by the interval between delivery and cell processing.[19] To date, at least three different kinds of stem cells have been isolated from UCB, including hematopoietic stem cells, endothelial progenitor cells, and MSCs.[18] MSCs revolve in cord blood but at much lower frequencies than hematopoietic stem cells,[10] which are $0.002 \pm 0.004 \times 10^6$ initially plated cells while the number of MSCs from BM is $83 \pm 61 \times 10^6$ initially

plated cells.[17] Thus, their isolation and culture are more difficult.[10] Generally, MSCs are successfully isolated from only 30% to 75% of UCB.[8,17,19]

The UCB is aspirated from the umbilical vein directly after the child's birth while the placenta is in utero and collected in a sterile bag containing anti-coagulant; citrate phosphate dextrose (CPD) or Acid citrate dextrose formula A (ACD-A) solution.[17] Any blood clot in the bag contributes to the exclusion of the bag.[19] Also, in the second trimester of pregnancy, the UCB can be collected transcervical after dilatation by using a cordocentesis with a 23-gauge needle, and kept in heparin.[22] Collected samples should be subjected to the following tests: complete blood count (CBC), bacteriological anaerobic and aerobic tests and immunological tests of antibodies for Herpes simplex virus type 1, 2 (HSV1, 2), Toxoplasmosis, hepatitis C virus (HCV), Hepatitis B surface antigen (HBsAg), and cytomegalovirus (CMV). Therefore, the following immunological tests were used for quality control of the samples including: Ac-HSV1+2 (IgM+IgG), HBsAg, Ac-HCV (IgM+IgG), Ac-anti Toxoplasmosis (IgM+IgG), and Ac anti-CMV (IgM+IgG).[21] By incubation in $NH_4Cl$ (8.4 g/L)/$KHO_3$ (1 g/L) buffer for 10 minutes at 4°C, then UCB is depleted from red blood cells. The most widely used cryoprotectant for banking of hematopoietic stem cells (HSC) and cord blood samples is DMSO. A cooling rate of 1–2ºC/min is suitable for most human cells, including HSC of UCB. It can be achieved by two conventional freezing methods including: controlled-rate freezing and uncontrolled-rate freezing or passive cooling. Recently, verification is used as an alternative technique for conventional methods. Viability of HSC, storage in −80ºC is decreased with the length of storage. Therefore, an ultra-low temperature is applied by direct immersion of sample bags in liquid nitrogen which has a constant temperature of −196ºC[23] or are freshly processed to obtain the mesenchymal stem cells.[17] Some researchers believe that the MSC isolation rate is not affected by cell processing protocol.[19] Ficoll,[19,24,25] hydroxyethyl starch,[19] and dextran[21] are the materials added to cord blood mononuclear cells to purify mononuclear cells.

However, density gradient centrifugation of 1.073 g/mL density compared to 1.077 g/mL density causes the greatest concentration of MSCs to 1.8-fold.[17] Ficoll and hydroxyethyl starch lead to 40–60%[10,26] and 50%[19] of purified mononuclear cells, respectively. In addition, flow cytometry (immunoselection) for sorting of CD34 cells, with a 32% success rate,[19] and the osmotic selection method are the other methods for MSC isolation from UCB.[17]

## Amniotic Fluid

Amniotic fluid (AF) MSCs express Oct-4, the pluripotent marker, in almost 90% of the favorable condition, also they have multiple differentiation capacities like the other mesenchymal stem cells.[11] Three types of adherent cells are characterized in AF. They are categorized based on their morphological growth and biochemical characteristics, which are as follows: 1) Epithelioid (E-type) cells are cuboidal to columnar cells derived from the foetal skin and urine; 2) Amniotic fluid (AF-type) cells originate from foetal membranes; and 3) Fibroblastic (F-type) cells are generated mainly from fibrous connective tissue. Both AF- and F-type cells demonstrated fibroblastoid morphology.

Human amniotic fluid stem cells (AFSCs) can be easily isolated from a small amount of sample during routine amnioptenteses in 14–16 weeks of pregnancy,[27] or they can be collected by puncture of the embryonic membranes during a caesarean section after opening the uterine wall. Amnion fluid should be processed within 4 h.[23] Filtering of AF through a cell strainer (40 µm strainer)[28,29] is the most common method for isolating MSCs, with a 25% success rate.[10,30] Also, immunoselection (a kind of cell sorting) with an antibody specific for c-Kit (CD117) antigen gives the total cell count of approximately $10^4$ to $10^6$ of cells per 5 mL of AF.[31,32]

## Human Placenta Tissues

Human placenta is made up of both foetal and maternal tissues, which are composed of amnion, chorion, and decidua.[33] The amnion and chorion have foetal origin, while the other parts of the decidua have maternal origin.[23] After fertilization the human blastocyst, composed of trophoblast and embryoblast, is embedded in the endometrium by invasion of the trophoblast cells.[33]

MSCs can be isolated approximately from all of the sections of the placenta.[34] It is shown that MSCs obtained from the first trimester of a preterm human placenta have higher proliferation potential than term human placenta cells and adult bone marrow. These findings may be due to the fact that the cells of early gestational placenta are closer to the embryo stage than the cells of late full-term placentas or adult bone marrow.[35]

## Amniotic Membrane

Amniotic membrane includes mesenchymal and epithelial cells. Human amniotic mesenchymal stem cells (hAMSCs) are derived from the extra embryonic mesoderm and are dispersed in the collagenous stroma, which is beneath the

epithelial monolayer of the amniotic membrane.[36] The amniotic cavity develops of endoderm, mesoderm, and ectoderm germ layers. The amniotic membrane lacks any vascular tissue and forms the majority of the inner layer of the foetal membrane, and is composed of three layers: 1) an outer mesenchymal cell layer; 2) an acellular intermediate basement layer, and 3) an epithelial monolayer consisting of epithelial cells. The outer layer is placed in close proximity to the chorion and has a lot of stem cells. Amniotic membrane stem cells (AMSCs) have two types, the amniotic epithelial stem cells and the amniotic MSCs, which are derived from the amniotic epithelial and the amniotic mesenchymal layers, respectively.[27]

Firstly, the amniotic membrane is mechanically separated from the chorion by detachment, it is rinsed in PBS or medium, minced with scissors, and then flushed via a 100-μm nylon filter,[23] or the minced pieces (1–2 mm$^3$) are transferred to a plate for explant culture for 10 days to allow the isolation and migration of the mesenchymal stem cells.[33] The minced amnion tissues are incubated with 0.25% trypsin at 37°C for 5 minutes to remove the epithelial cells (EC). Then, by various types and concentrations of collagenase with or without adding DNAse, the remnant tissues are digested and the mesenchymal stem cells are released.[36] It was reported that using 1 mg/mL collagenase type I or IV[26,37] with 20–75 μg/mL DNAse,[36] or 4 mg/mL collagenase type II with 100 μg/mL DNAse, is sufficient for cell extraction.[38] Nevertheless, the incubation times can vary from 30 minutes to 3 hours.[36] It was shown that isolation efficacy or harvesting rate with collagenase is 62.5–100%.[10] The yield from term amnion is about 1 million MSCs per gram of amnion tissue;[39] approximately 1×10$^6$ cell/g of tissue.[10] But some groups have treated cells with dispase and papain instead of DNase or pure MSC without the previous isolation of EC by treatment with trypsin.[36]

## Chorionic Membrane

Chorionic membrane (plate) contains chorionic trophoblastic cells and MSCs.[10] Chorionic MSCs are derived from the reticular layer of the chorion. So, for obtaining them, chorion is separated from the amnion by peeling it apart. As collagen is the major part of the ECM of chorion layers, so collagenase mediated chorion dissociation is a crucial step in all types of cell isolation procedures. The minced chorion fragments were exposed to 0.25% or 0.5% trypsin-EDTA, and then to complete the tissue digestion 0.5–3 mg/mL collagenase (I) was applied for 20–30 minutes.[35,40] Also, it was reported that combinations of mechanical and enzymatic treatments with dispase and/or collagenase IV can be used for cell isolation from the chorionic membrane.[10,41]

## Decidua

The decidua is composed of a thin layer of maternal endometrial tissue which is reformed by structural and functional transformation during early pregnancy.[42] Decidua basalis is the attachment site of the placenta and endometrium, while decidua parietalis refers to the other parts except the attachment site of the placenta. Cells originating from these parts are very different in their function.[43]

Decidua basalis and decidua parietalis can be collected by scraping them from the chorion. Tissue parts are then chopped and minced before being passed through a filter[23] or explanted in dish culture for 10 days until the cells migrate from the decidua.[33] In addition to mechanical methods, enzymatic methods are also used for isolating the cells from the decidua, based on collagenase usage. Until now, collagenase type I and IV with trypsin, DNase, and dispase have been successfully used for the digestion of the decidua. In enzymatic method, decidua basalis is dissected from the central region of the placenta and mechanically minced into 1–2 cm$^3$ pieces then digested with 0.25% trypsin and 1 mg/mL collagenase IV incubated for 30 minutes at 37°C.[23,42] Also, the minced tissues can be digested using a solution containing 3 mg/mL collagenase type I and 271 units/mL DNase I at 37°C for 1 hour.[43] The tissues can also be digested in 0.25% trypsin and 50 mg/mL DNAse 1 at 4°C overnight. Then, after inactivating the trypsin with FBS, the remaining tissues are treated with 10 mg/mL type 1 collagenase and 50 mg/mL DNAse I for 30 minutes at 37°C. In this way, at the first passage when the population displays a homogeneous fibroblast-like morphology, about 5×10$^5$ cells from an initial 8 g of tissues were obtained.[44] Also, a combination of 100 U/mL collagenase type I, 1.5 μg/mL DNase I, and 2.4 U/mL dispase in serum-free DMEM can be used for 1.5 to 2 hours, while every 30 minutes the cocktail is gently agitated for 10 seconds.[45]

## Chorionic Villi

The number of cells that can be obtained from chorionic villi specimens is limited due to their small amount of tissue and short-time culture.[46] For this purpose, amnion and chorionic membranes are removed from the placenta then white terminal chorionic villi from a central cotyledon of the villous

vascular floor are cut into small pieces and explant culture continues for 2 or 3 weeks until the cells are observed.[47] Nevertheless, in the explant culture, contamination with maternal tissues is more likely.[48] Also, in another method, the chorionic villi pieces can be hemolyzed in red blood cell lysis buffer (155 mM $NH_4Cl$, 10 mM $KHCO_3$, 0.1 mM EDTA, pH 8.0), centrifuged at 550 g for 5 minutes at room temperature, and then treated twice with 0.25% or 0.05% trypsin-EDTA in a magnetic stirrer at 37°C for 10 minutes.[49,50] Besides, the chorionic villi can be digested in 2.5% trypsin and 271 unit/mL DNase, with gentle rotation at 4°C overnight. Then, the tissues are allowed to adhere to the plastic floor of six-well plates for 60 minutes. Supplemented medium is then gently added to the tissues and cultured for 14 days, with refreshing every 3 days. The MSCs migrate out of trypsin-treated tissues due to digestion of the ECM. Results showed that trypsin significantly increases the number of cells produced by the explanted chronic villi as compared to untreated villi. It is reported that there are $11.55 \times 10^3$ and $24.66 \times 10^3$ cells produced per 40 mg of untreated- and treated tissues, respectively.[51] Also, it showed that 1 mg/mL collagenase type I at 37°C for 1 hour was able to separate enough cells.[46]

## Umbilical Cord (UC)

The UC contains two arteries and one vein surrounded by Wharton's jelly (WJ), matrix, and connective tissue of the UC, which is rich in proteoglycans and hyaluronic acid. It is revealed that MSCs from UC contain two subpopulations of 11 and 19 μm average diameter. The small-sized subpopulation expresses a higher level of CD73 and CD90 and exhibits higher proliferative capacity.[17]

UC can even be freshly processed for as long as 5 days for later cutting while leading to a large enough number of cells, but usually it is processed between 1–24 hours. It should be noted that 2–6 hours is the best interval time from cutting to processing of the umbilical cord.[17] Also, after removing its vessels and mincing into 0.5–1 cm sections, the UC can be cryopreserved in 10% DMSO for future use.[52]

## Wharton's Jelly

Wharton's jelly (WJ) is a mucoid connective tissue located around the vessels of the UC.[36] There are several methods to isolate the cells from Wharton's jelly. In an easy method, UC vessels are separated and Wharton's jelly tissue is minced and placed in PBS for 1 hour with stirring. After discarding the remnant tissues, the remaining

suspension is centrifuged and the pellet is cultured.[53] Also, the minced pieces are placed into the dish during 14 days for explant culture.[4,54] WJ pieces can be digested by 2 mg/mL collagenase for 16 hours and then suspended in 2.5% trypsin for 30 minutes,[55] or in 75 μg/mL (0.075%) collagenase type II for 30 minutes then in 0.125% trypsin for 30 minutes[56] and 1 mg/mL collagenase type B for 3 hours then 0.25% trypsin for 15 minutes with gentle agitation at 37°C. To date, different enzymatic dosages, types, and times have been examined for obtaining MSCs from WJ. By these methods, the initial cell number is $0.5–1 \times 10^4$ cells per cm of UC and $1 \times 10^5$ cells per gram of WJ.[57] Furthermore, WJ can be digested in 1 mg/mL collagenase type B and 0.3 mg/mL hyaluronidase for 2 hours, followed by 0.25% trypsin-EDTA for 15 minutes at 37°C.[57] This method leads to the initial cell number of $0.25–5 \times 10^4$ cells/cm of UC and $0.5 \times 10^5$ cells/gram of WJ.[10,57] Also, solution containing collagenase type I, collagenase type IV, and 100 IU of hyaluronidase at 37°C for 45 minutes is used for digestion of the WJ. Through the enzymatic method, it should be separated by the blunt surface of forceps and passed through an 18 G needle for better digestion. The number of isolated cells by this method was $4.7 \times 10^6$ live cells/$cm^2$.[56,58,59] The combination of enzymatic and explant methods can lead to good results. The dissected WJ are digested in 4 mg/mL collagenase type I and 0.3–1 mg/mL hyaluronidase for 1 h, followed by 0.1% trypsin-EDTA for 30 minutes at 37°C. Then, WJ pieces are centrifuged and in addition to the cell pellets, the undigested WJ pieces are also cultured in supplemented medium until migration of MSCs.[60]

## Umbilical Cord Vein

UC vessels have both endothelial and mast cells (MCs). For separating MCs, after immersing the whole cord in 70% ethanol for 30 seconds and washing it in PBS, it is dissected to obtain a vein. The vein is minced into small pieces (2 $mm^3$) and is explanted with supplemented medium. Tissue explants are removed after 21 days of culture.[61] For enzymatic method, the umbilical vein is cannulated with a catheter and ligated with a cardiac cotton tape and washed internally with PBS or PBS containing 100 μg/mL heparin (PBS-H). Then it is clamped at one end and perfused with 10 mL of 1–4 mg/mL collagenase type I or IV at 37°C for 20–60 minutes. During incubation, cord walls are occasionally massaged to promote enzymatic action via the pressure on underlying UC tissues. Then the clamps are released and the collagenase-containing solution from

**Table 1** Brief Conclusions of This Review

| Sources | Methods | The Most Effective Method(s) | The Easiest Method(s) | Reference |
|---|---|---|---|---|
| Cord blood | 1. Ficoll+Centrifuge<br>2. Hydroxyethyl Starch+Centrifuge<br>3. Dextrane+Centrifuge<br>4. Immunoselection<br>5. Osmotic Selection Method | 1, 4 | 4 | 17,19,21 |
| Amniotic fluid | 1. Filtering+Centrifuge<br>2. Immunoselection | 2 | 1 | 29,31 |
| Amniotic membrane | 1. Mincing+Flushing<br>2. Explant<br>3. Collagenase (type Ⅱ)+DNase<br>4. Trypsin+Collagenase (type I or IV) or/and DNase | 3 | 1 | 32,35,37 |
| Chorionic membrane | 1. Trypsin+Collagenase (type I)<br>2. Dispase+Collagenase (type IV) | 1, 2 | 1, 2 | 10,34,39,40 |
| Decidua | 1. Mincing+Flushing<br>2. Explant<br>3. Trypsin+Collagenase (type IV)<br>4. Collagenase (type I)+DNase<br>5. Trypsin+DNAse+Collagenase (type I)<br>6. Collagenase (type I)+DNase+Dispase | 5 | 1 | 32,42,44 |
| Chorionic villi | 1. Explant<br>2. Trypsin-EDTA<br>3. Trypsin+Explant<br>4. Collagenase (type I) | 3 | 1 | 45,47,49,50 |
| Wharton jelly | 1. Stirring in PBS<br>2. Explant<br>3. Trypsin+Collagenase<br>4. Collagenase (type B)+Hyaluronidase+Trypsin<br>5. Collagenase Type I+Collagenase Type IV +Hyaluronidase<br>6. Enzymatic+Explant | 5 | 1 | 4,52,56,59 |
| Umbilical cord vein | 1. Explant<br>2. Collagenase (type I or IV) | 1 | 2 | 60,63 |
| Umbilical cord arteries | 1. Explant<br>2. Collagenase | 1 | 2 | 17,60 |
| Umbilical cord lining cells | 1. Explant | — | — | 15 |
| Whole umbilical cord | 1. Explant<br>2. Collagenase | 1 | 2 | 60 |

inside the vein is collected. After centrifugation, the cell pellet is cultivable.[62,64] It should be noted that with using 0.25% trypsin for 15 minutes at 37°C only, endothelial cells will be separated from the umbilical vein.[65]

## Umbilical Cord Arteries
The whole cord is immersed in 70% ethanol for 30 seconds, then immediately washed in PBS. After obtaining the arteries and mincing them into small pieces (1–2 mm$^3$), they are

explanted for 21 days until the migration of the fibroblast-like adherent cells from the fragments,[61,66] or a 4–5 cm length of the artery is isolated from the surrounding matrix and the two ends are ligated with a surgical suture to form a loop. Then, it is filled with 1 mg/mL collagenase and incubated at 37°C for 15 minutes[62,67] or even for 18–24 hours.[59] Some studies have reported that with open-ended vessels, the cells can be isolated so it may not be necessary to seal the two ends of the blood vessel, and no signs of contamination are observed in the isolated cells.[17]

### Umbilical Cord Lining Membrane
In fact, UC lining membrane is sub-amniotic of the cord. It has two types of cells, MSCs, and epithelial cells. The MSCs can be separated from the sub-amniotic substrate and epithelial cells can be isolated from the amniotic layer. Also MSCs can be isolated by the explant method, as explained before for the other cells.[15]

### Whole Umbilical Cord
In general, MSCs can be isolated from the sub-endothelium, the WJ, the perivascular region, and the vessels.[36] But, when the whole UC is digested by enzymes, the cell population obtained by this method may contain endothelial, epithelial, and MCs.[17]

After being dissected and rinsed in 75% ethanol, the whole cord is explanted.[61,68] If the enzyme should be used, small pieces of the cord are digested by 1.25 mg/mL collagenase XI for 1.5 hours,[69] or 1 mg/mL collagenase I for 1 hour, and then incubated in the incubator-shaker at 37°C.[61]

## Conclusion and Remark
Taken together, due to the foetal source of extra embryonic tissues and their isolated MSCs, they have specific properties including high proliferative capacity and expansion potential. Thus, the suitable isolation process can reduce the time and passage number. In this way, a commonly used method for MSC isolation from extra embryonic sources is enzymatic digestion employing collagenase, DNase, dispase, and trypsin. However, each tissue has special characteristics that should be taken into consideration during the isolation procedure to get a considerable number of cells. Table 1 shows briefly the conclusions of this review.

## Disclosure
The authors report no conflicts of interest in this work.

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