Explant culture: An advantageous method for isolation of mesenchymal stem cells from human tissues

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Abstract

Mesenchymal stem cell (MSC) research progressively moves towards clinical phases. Accordingly, a wide range of different procedures were presented in the literature for MSC isolation from human tissues; however, there is not yet any close focus on the details to offer precise information for best method selection. Choosing a proper isolation method is a critical step in obtaining cells with optimal quality and yield in companion with clinical and economical considerations. In this concern, current review widely discusses advantages of omitting proteolysis step in isolation process and presence of tissue pieces in primary culture of MSCs, including removal of lytic stress on cells, reduction of in vivo to in vitro transition stress for migrated/isolated cells, reduction of price, processing time and labour, removal of viral contamination risk, and addition of supporting functions of extracellular matrix and released growth factors from tissue explant. In next sections, it provides an overall report of technical highlights and molecular events of explant culture method for isolation of MSCs from human tissues including adipose tissue, bone marrow, dental pulp, hair follicle, cornea, umbilical cord and placenta. Focusing on informative collection of molecular and methodological data about explant methods can make it easy for researchers to choose an optimal method for their experiments/clinical studies and also stimulate them to investigate and optimize more efficient procedures according to clinical and economical benefits.

1 | INTRODUCTION

Mesenchymal stem cell (MSC) research progressively moves towards clinical phases due to their beneficial characteristics such as stemness and plasticity, lack of alloreactivity and lack of acute adverse reaction.^{1–4} One of the most important steps in MSC therapy is their preparation prior to their administration and the first step of preparation is isolation from original tissue. Increasing use of human adult and perinatal tissues for MSC isolation for clinical applications necessitates paying more attention to selection of a proper isolation method. A systematic search in PubMed using

search key word “mesenchymal stem cell” and activated filter “clinical trial” obtained 286 results from which 203 were human clinical studies including more than three patients. Statistical analysis of these studies showed application of MSCs in diverse clinical conditions (Figure 1A) and also growing interest towards use of non-bone marrow human tissues as MSC sources (Figure 1B). MSCs have been isolated from various human tissues such as bone marrow (BM),⁵ adipose tissue (AD),⁶ dental pulp (DP),⁷ hair follicle (HF),⁸ cornea,⁹ umbilical cord (UC)¹⁰ and placenta.¹¹ Isolation methods were categorized into two major techniques; enzymatic methods and explant culture method. In enzymatic methods, one, two or in

Abbreviations: MSC, mesenchymal stem cell; AD, adipose tissue; BM, bone marrow; DP, dental pulp; HF, hair follicle; UC, umbilical cord; WJ, Wharton's jelly; ASC, adipose-derived stem cells; SVF, stromal vascular fraction; DMEM, Dulbecco's modified Eagle's medium; α -MEM, minimum essential medium- α ; RPMI, Roswell Park Memorial Institute medium; PBS, phosphate-buffered saline; FBS, foetal bovine serum; FCS, foetal calf serum; EDTA, ethylenediaminetetraacetic acid; ECM, extracellular matrix; PG, proteoglycan; CK, cytokine; GF, growth factor; VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; IGF, insulin-like growth factor; EGF, epidermal growth factor; HGF, hepatocyte growth factor; PDGF, platelet-derived growth factor; KGF, keratinocyte growth factor; TGF β , transforming growth factor β ; IL, interleukin; CCL, chemokine (CC motif) ligand; CXCL, chemokine (C-X-C motif) ligand; HIV, human immunodeficiency virus; PCR, polymerase chain reaction; PBMC, peripheral blood mononuclear cell; MLR, mixed lymphocyte reaction.

FIGURE 1 Statistical analysis of mesenchymal stem cell clinical studies published in PubMed until June 2016. A, MSCs have been applied in diverse clinical conditions, mostly including cardiovascular diseases, transplant-related conditions, neurologic disorders, bone, joint, gingival disorders and dental implant, and liver complications (n=203). B, Application of adult and perinatal tissues other than bone marrow has been increased in clinical studies during recent years (N=196)

some procedures three proteolytic enzymes are used for separation of cells from tissue; the single cell suspension is then cultured in appropriate medium for further cell proliferation. On the other hand in explant method, no enzyme is used; original tissue is excised into smaller pieces which are placed in culture dishes or flasks, and cells then start to migrate out of tissue and adhere to the culture surface.¹² These two techniques are different by many aspects such as time to reach isolated cells and economy; however in the case of MSC isolation, it seems that both of them provide acceptable cell yield.^{13,14} Explant method possesses several advantages for MSC isolation which is not clearly described in the literature till now. These advantages can make this method as the best choice for MSC isolation in many research and clinical projects. In this review, I focus on molecular events and methodological aspects of explant culture technique for MSC isolation from human adult and perinatal tissues to provide a useful reference for researchers who are going to choose a suitable tissue source and a proper method for MSC isolation, and also for researchers who are interested in optimizing MSC isolation methods. To gather current applicable information, a research project was undertaken to build main parts of the targeted plan: First, advantages of the explant method including presence of the companion tissue in first steps of primary culture and absence of proteolytic enzymes were discussed. Second, explant isolation methods from most common adult (AD, bone marrow, DP, HF and cornea) and perinatal (UC and placenta) MSC sources were searched and were presented in their most intact form that was provided by the researches from the original articles.

2 | ADVANTAGES OF PRESENCE OF EXPLANT TISSUE PIECES IN PRIMARY CULTURE

In explant culture method, the tissue piece is present during primary culture. The companion of the tissue piece provides some advantages for migrating cells. Although attachment of cells to extracellular matrix

(ECM) is not dissociated due to absence of proteolytic enzymes and this is the reason for longer isolation time period; its advantages are not noticed usually. The tissue consists of ECM and cellular components and both of them are active during the process of primary culture (Figure 2) and can exert several advantages as will be discussed below; however, presence of other cell types cannot be troublesome for obtaining pure MSC population because it is limited to primary culture phase. Indeed, unattached cells such as hematopoietic stem cells (in bone marrow sample) will be gradually removed after refreshing the culture media. Adherent non-stem cells will also be omitted during first subcultures due to their limited ability to proliferate and produce same cells.

2.1 | Extracellular matrix continues to provide some benefit

In addition to physical scaffolding for the cellular constituents, ECM also provides crucial biochemical and biomechanical signals that are essential for tissue morphogenesis, differentiation and homeostasis. ECM receptors, such as integrins, discoidin domain receptors and syndecans,^{15–17} mediate cytoskeletal coupling to the ECM and involve in cell migration through the ECM.¹⁸ On the other hand, the ECM constantly remodels and acts dynamically by modification of its molecular components post-translationally.

Many ECM proteins, including laminins, tenascins, thrombospondins and fibrillins, contain embedded EGF-like motifs. These structures may bind as anchored ligands to EGF receptors on cells and trigger signals in such a way that is induced by soluble ligands.^{16,19,20} ECM is also a localized reservoir of growth factors and cytokines. Collagens, proteoglycans (PGs), fibronectin, and vitronectin, themselves, or in combination with heparin and heparin sulphate, bind many growth factors, such as fibroblast growth factors (FGFs) and hepatocyte growth factor (HGF);²¹ they regulate growth factor bioavailability by establishing stable gradients.¹⁶ Local release of soluble growth factors from their insoluble anchorage can be induced following proteolytic degradation of ECM

FIGURE 2 Advantages of explant method comparing with enzyme digestion for MSC isolation. A, Enzymatic digestion method for MSC isolation: tissue fragments are treated with proteolytic enzyme(s) to obtain a single cell suspension. After washing steps, single cell suspension is cultured in the culture dish. During this process, cells are completely separated from tissue and endogenous CKs and GFs are also washed away. Therefore, cells are transferred to the culture dish after two types of stress; proteolysis and separation from the tissue (all of its physiological communications are cut suddenly), and without addition of exogenous GFs, risk of failure is too high. B, Explant culture for MSC isolation: tissue piece(s) is placed in the culture dish without any digestion step. Cells are migrating out of the tissue due to filling of wound condition. They are communicating with companion tissue and cells. Continuation of molecular communication between migrating cells and tissue piece (physiological type of signals and interactions) reduces stress of primary culture; released CKs and GFs from ECM reservoir and cells (of the tissue explant) stimulate cell growth and proliferation without requirement for addition of any exogenous growth factor

proteins or PGs by enzymes such as metalloproteinases²² (evidences for growth factor content of tissues will be discussed widely in the following parts).

Interactions between ECM proteins and growth factors with cell surface receptors stimulate cellular responses through signal transduction and gene transcription regulation. These physical and biochemical characteristics represent a functional model in which the ECM mediates protection by a buffering action and maintains extracellular homeostasis and water retention.^{22,23}

Stem cell niche plays key roles in stem cell fate²⁴ and ECM is an effective stem cell niche component. Soluble and matrix-bound effector molecules of ECM interact with cells and regulate their functions and homeostasis between self-renewal and differentiation.²⁵ Actually extrinsic cues which are provided by the niches' unique microenvironment and supportive ECM, maintain stemness of stem cells, determine their proliferation rate and specify whether to divide asymmetrically or symmetrically.²⁴ Reduction of instructive cues in injured and aged ECM

affects stem cell behaviour.²⁶ Slight variations in ECM characteristics such as altered protein biosynthesis, post-synthetic modifications or imbalanced proteolytic degradation of ECM components influence stem cell self-renewal potential.^{27,28} Following tissue damage and vascular injury, a wound response stimulates monocyte infiltration to the damaged ECM. Degradation products and cytokines bind to ECM, and monocytes rapidly differentiate into macrophages.²⁹ These activated macrophages, then, secrete several growth factors, cytokines and matrix metalloproteinases.³⁰ The remodelled ECM promotes directional migration of cells towards site of injury.³¹ In explant method, it is expected that wound response proceeds; cytokines and enzymes are released, and stem cells migrate towards injured parts and out of the tissue. It seems that in explant method, cells start to migrate out of tissue in a process like wound healing and they gradually exit the tissue while they keep their interaction with the tissue explant. If cell migration from tissue pieces occurs in a similar way to wound healing process, it is better to make incisions in the explant tissue piece to facilitate the migration process (Figure 2).

2.2 | Cytokines and growth factors are released from the tissue pieces

As discussed earlier, the tissue injury triggers production and release of growth factors by cells in the injured tissue. Therefore, explant tissue pieces release growth factors to the medium due to the activation of wound healing processes in the explant tissues. The tissue offers a cocktail of cytokines and growth factors (not only one, two or three) which can be very expensive when they are added exogenously, especially in large scale. On the other hand, migrated cells may continue their communication with original tissue piece and transduce signals to regulate secretory events in favour of their requirements; therefore, these cytokines and growth factors may be released from the tissue pieces in proper concentrations which are required by migrated cells. The mutual paracrine relationship between migratory cells and tissue piece may continue for a limited time period.

On the other hand, after enzymatic isolation of MSCs, most of the researchers add growth factors as supplement to media during all steps of cell growth and proliferation. They mostly use β -FGF and platelet-derived growth factor (PDGF).³²⁻³⁷ However, in explant culture, the tissue piece can play as a growth factor reservoir during the primary culture.

In this part, I focus on evidences for cytokine and growth factor content of the most common MSC tissue sources (Figure 3).

2.2.1 | Adipose tissue

Adipose tissue is a source of growth factors such as β -FGF and vascular endothelial growth factor (VEGF).^{38,39} Adipocytes and resident macrophages are the major sources of VEGF in AD.^{40,41} Gabrielsson et al. investigated the expression of several FGFs and FGF-receptors

(FGFRs) in human AD and adipose tissue cell fractions obtained from both subcutaneous (sc) and omental (om) depots and detected FGF-1, FGF-2 (or β -FGF), FGF-7, FGF-9, FGF-10 and FGF-18 transcripts. They found similar expression of FGF-2, FGF-7 and FGF-10 in sc and om AD, and higher levels of FGF-1 and FGF-9 in the om AD. Adipocyte and stroma-vascular (SV) fractions were prepared from both depots and analysed separately for growth factor expression. FGF-2 was only expressed in adipocytes; FGF-7, FGF-9 and FGF-18 were expressed in the SV fraction; and FGF-1 and FGF-10 were expressed in both adipocytes and the SV fraction.⁴² Currently, the most abundant growth factor that is added to MSC culture medium is FGF-2 or β -FGF; therefore, adipocytes can support MSCs' growth at least by releasing β -FGF in the primary steps of in vitro culture, and their presence seems to be supportive. Indeed, the presence of SV fraction is not enough for secretory support of MSCs growth and presence of adipocytes in addition to SV fraction in the explant culture can improve in vitro media for stem cell growth and proliferation.

2.2.2 | Bone marrow

Bone marrow aspirate contains large quantities of growth factors, cytokines, ECM molecules and cell-adhesion molecules involving in osteogenesis, hematogenesis, neural growth and angiogenesis.^{43,44} Bone marrow nucleated cells secrete angiogenin, VEGF, HGF, insulin-like growth factor 1 (IGF-1), transforming growth factors (TGFs) interleukin 10, chemokine (C-C motif) ligand 2 (CCL2), CCL23 and CCL24, chemokine (C-X-C motif) ligand 6 (CXCL6), CXCL12 and CXCL13, and FGF9.^{45,46} Specifically, BM-MSCs were found to secrete many growth factors and cytokines and perform their actions through paracrine and autocrine effect, this characteristic is also of importance in cell therapy purposes such as cardiac and neurologic disorders.⁴⁷ Secretions from

FIGURE 3 Growth factor and cytokine content of human tissues from which mesenchymal stem cells are commonly isolated. Tissues which are used as MSC source produce growth factors and cytokines and store them in the extracellular matrix. These GFs and CKs can be released into the culture medium of the explants primary culture and stimulate growth and proliferation of migrated cells

MSCs and other BM cells are present in the aspirated sample and can be very effective in first steps of in vitro cell isolation and primary culture and aid easier adaptation of isolated cells with new environment.

2.2.3 | Dental pulp

Growth factors such as VEGF, FGF-2, PDGF, TGF- β and HGF were isolated from human DP tissue,^{48,49} and they were also found in pulp fibroblast supernatant.^{50,51} Both the physiological cell populations of the pulp and the inflammatory cells, which locally infiltrate the tissue at sites of injury, express several growth factors.⁵² Against dentin matrix in which these growth factors become "fossilized", in the ECM of the pulp soft tissue, they are in greater turnover, and contribute to the more immediate cellular responses.⁵² Dentin matrix contains a mixture of cytokines and growth factors which are probably produced by odontoblast cells; they may be released into the pulp environment during tissue injury⁵³ and to the media during primary explant culture of pulp stem cells.

2.2.4 | Hair follicle

Production of new hair is a cyclic process in which follicles produce an entire hair shaft from tip to root. When follicle stem cells receive signals through growth factors and cytokines, the next growth phase starts.⁵⁴ Many growth factors, such as keratinocyte growth factor (KGF) and various FGFs, are released by mesenchymal cells and act as strong mitogens for other follicular cells.⁵⁵⁻⁵⁷ Kozłowska et al.⁵⁸ found that cultured follicular cells strongly expressed mRNA of four VEGF molecular species. Other growth factors, such as HGF,⁵⁹ PDGF,⁶⁰ FGFs1, 2, 5, 7, 10, 13, 22 and epidermal growth factor (EGF),⁶¹⁻⁶³ are shown to be expressed in dermal and hair follicular cells to regulate hair growth and skin regeneration.

2.2.5 | Cornea

Maintaining homeostasis and normal corneal function and also wound healing response in human cornea is regulated by a number of growth factors and cytokines which is produced by the corneal epithelium, stroma and endothelium.^{64,65} Evidences established production of EGF,⁶⁶ TGF- β ⁶⁷ and β -FGF⁶⁸ by three corneal cell types (stroma, endothelial and epithelial cells). KGF and HGF are believed to be produced by corneal fibroblasts. PDGF is found mainly in the epithelium, IGF in the corneal epithelium and stroma, and the trabecular, meshwork, high levels of aFGF in Bowman's and Descemet's membranes and in the corneal endothelium, and bone morphogenic proteins in all layers of cornea. Nerve growth factor, neurotrophin-3 and -4 (NT-3 and -4), and glial cell-line-derived neurotrophic factor (GDNF) and (VEGF) have been detected in some studies in the corneal tissue.^{64,69}

2.2.6 | Umbilical cord

Umbilical cord tissue is a source of cytokines and growth factors such as β -FGF, PDGF, EGF, TGF- β and IGF-1. The most abundant

is IGF-1 (almost 350 ng per 1 g of Wharton's jelly). TGF- β , α -FGF and β -FGF also exist in nanograms; however, their amounts are distinctly lower. PDGF and EGF exist in picogram concentrations. The amount of these cytokines in the Wharton's jelly tissue is comparable with or higher than the amount, which exist in the wall of UC artery. Sobolewski et al.⁷⁰ founded that β -FGF was released from tissue pieces during primary phase of explant culture in the medium.

2.2.7 | Placenta

Implantation and growth of the placenta requires extensive angiogenesis and vascular transformation to establish the structures involved in exchange. For this purpose, growth factors and cytokines are produced and secreted in placenta tissue, which were detected in several investigations. The presence of four species of mRNA encoding VEGF was demonstrated in both first trimester and term placenta.^{71,72} VEGF was found to be produced by cells within the villous mesenchyme, decidual macrophages and decidual glands.⁷³ FGF-2 is also expressed in first trimester and term placenta, its localization was detected in the syncytiotrophoblast surrounding the placental villi and in/around cytotrophoblast cells in first trimester placenta and in the syncytiotrophoblast and foetal membranes at term. FGF-2 mRNA is localized strongly in the smooth muscle cells around the mid- and large-sized placental vessels.^{74,75} Other growth factors are also produced in placenta tissue⁷⁶ including PDGF in cotyledons,^{77,78} HGF in the villous core compartment,⁷⁹ EGF in cytotrophoblast and syncytiotrophoblast,⁸⁰ IGF-1 in cytotrophoblast and syncytiotrophoblast,⁸¹ and IGF-2 in cytotrophoblast.⁸²

3 | ABSENCE OF PROTEOLYTIC ENZYMES IN ISOLATION PROCESS

3.1 | No enzyme/proteolytic stress on cells

Sutradhar et al. showed that trypsin-EDTA mixture induced a significant loss of both membrane integrity and cell viability in a time-dependent manner; they investigated most commonly used concentration of trypsin-EDTA (0.25% trypsin with 0.02% EDTA) on equine chondrocytes (for 5, 20 and 60 minutes) and considered the 5-minute time period as control. They found that after 20 and 60 minutes of trypsinization, the cell membranes were strongly affected and the percentages of viable cells reduced to 91% and 85% respectively. They suggested that as minimum trypsin exposure time as possible should be applied in the process of cell isolation from tissue.⁸³ Therefore, it is necessary to carefully set up the process of enzymatic cell isolation which may be time-consuming at first.

Trypsinization can also affect cell surface antigens,⁸⁴ cell surface topography, cytoskeletal components and intra-membranous particle distribution.⁸⁵ These enzyme's toxic effects may revert during several days of culture⁸⁶; however, in primary culture, it may enhance the probability of isolation failure.

These effects are variable for different tissue samples in each isolation run due to either variability of lytic activity between batches of enzyme and variable sensitivity of tissues (from different persons) to collagenases; therefore, verification of enzymatic isolation method for clinical use is difficult.⁸⁷

In addition, in explant technique, cells are transferred from *in vivo* into *in vitro* condition in companion with a fragment of related tissue; actually they do not suddenly separate from their previous stable environment. This may help cells tolerate isolation stress better; in other words, in enzymatic isolation technique, cells are exposed to dual stress: separation from body and enzymatic interruption. Otte et al.⁸⁸ demonstrated that in the presence of the originating tissue pieces, the stem cell characteristics of MSCs maintained during long-term *in vitro* culture. They suggested that the related tissue fragments provides a microenvironment in which, MSCs retain in their stem cell-like state.

3.2 | No risk of viral transduction through enzyme product

Porcine trypsin is produced using huge numbers of pancreases; in this process, one infected animal may contaminate an entire batch. Although much of bovine serum and trypsin which are used in production of human biologicals are often subjected to virus removal and inactivation procedures, the sensitivity of current tests may not be sufficient to detect diluted contaminants. When sensitivity of tests is not enough for detection of low-level initial contamination, it can be amplified during the long course of scale-up. In addition, some viruses—that may have human host—may not be detected by the standard tests and may be of concern as contaminants.⁸⁹

Pinheiro de Oliveira et al. performed five PCR protocols to assess the presence of genetic material from mycoplasma, porcine circovirus 1, bovine leukaemia virus or bovine viral diarrhoea virus in cell culture samples and demonstrated that sera and trypsin (which were used by different laboratories) showed high rate of contamination. Their results highlighted the necessity of monitoring and controlling of biological contaminants in laboratories and cell banks which are working with these materials.⁹⁰

3.3 | Reduced price

Purchase of enzyme products raises the price in cell isolation experiment (in enzymatic isolation method). In addition, as it was described earlier, explant tissue pieces release cytokines and growth factors into the medium, which lead to cell growth stimulation; so there is no need to add any growth factor. Consequently, simple medium is again more cost-effective for MSC expansion in both research and clinical settings.

The process of set-up and design of explant culture method is also less labour-intensive and more cost-effective in comparison with enzymatic digestion, so more suitable for clinical purposes.

These facts are of special importance when large-scale application in clinical settings is the main objective.

4 | TECHNICAL HIGHLIGHTS OF EXPLANT ISOLATION METHOD FOR HUMAN MSCS

It is necessary to remind that before any human sampling, ethical aspects of the process must be completely achieved. In this part, pivotal technical points of MSC explant isolation from various sources are discussed and fundamental steps of explant primary culture methods for MSC isolation from human tissues are presented in Table 1.

4.1 | Adipose tissue

Lipoaspirate tissue and resected AD are appropriate sources of human MSCs. They can be collected from voluntary liposuction and abdominoplasty procedures. Priya et al. could obtain MSCs from both lipoaspirate and fat tissue using explant primary culture method. Cell populations in P0 were enriched in adipose-derived stem cells (ASCs), because the migration of plastic adherent cells from the tissue pieces served as the criterion for isolation and led to significantly reduced haematopoietic cells and contaminants.⁹¹ Ghorbani et al.⁹² isolated MSCs from AD slightly different; they washed fat tissue pieces with FBS before plating, and then incubated the explanted tissues with very small amount (about 50 μ L) of FBS for 24 hours. It seems that this step was performed for better tissue attachment. Jing et al. also isolated MSCs from inguinal fat pads of BALB/c mice. Their procedure was similar with little differences; they stated that tissue pieces should be placed with 5 mm space from each other and after placing them in the flask a 5-minute period is necessary for tissue attachment before addition of the medium. This time was much shorter than that for other tissues such as mitral valve and skin. This was probably because the released lipids made the minced AD sticky and easy to attach to the flasks.⁹³

In the case of lipoaspirate specimen, fat fraction can be processed by traditional methods; however, blood/saline fraction can also be used to isolate MSCs using a procedure that was performed by Francis et al. They presented a quick method for MSC isolation and expansion from lipoaspirate in <30 minutes, and used only standard tissue culture materials and equipment without using collagenase digestion and Percoll gradients, or multiple washings. They achieved an abundant population of adipose-derived MSCs with differentiation potential, characteristic cell surface markers and proliferative lifespan mostly same as MSCs extracted from bone marrow (BMSCs) or enzymatically processed ASCs.⁹⁴

4.2 | Bone marrow

The common method for MSC isolation from bone marrow is density gradient centrifugation which was discussed widely in many published studies and is not targeted to be explained again here; however, another method was also examined by researchers in which, the whole tissue was directly plated for primary culture.⁹⁵ In this method, it is necessary to prepare a single cell suspension by pipetting before plating of sample. Al-Qaisy et al. compared three methods for MSC isolation from BM and examined direct plating method. They stated

that in direct plating, primary tissue culture plate can be contaminated heavily with hematopoietic progenitors such as macrophages and fibroblasts; however, they tried to solve this problem by first using RPMI-1640 medium which inhibited the growth of hematopoietic cells in cultures, and second frequent media refreshing in 6 and 24 hours of initial culture.⁵

4.3 | Dental pulp

Once deciding to use teeth for the purpose of MSC isolation,^{96,97} possibility of contamination should be strictly considered. Laino et al. stated two valuable points in this relation: (i) checking for systemic and oral diseases and pre-treating a week before with professional dental hygiene, and (ii) covering the dental crown with a 0.3% chlorhexidine gel for 2 minutes, before extraction.⁹⁸ Perry et al. also recommended to transport the tooth on ice to the laboratory. They also provided a standard washing procedure before starting cell isolation: several washes of tooth in sterile PBS, followed by immersion in 1% povidoneiodine for 2 minutes, then immersion in 0.1% sodium thiosulfate in PBS for 1 minute, and another wash in sterile PBS.⁹⁹

To remove pulp tissue, the tooth should be broken carefully to avoid pulp damage. For the best, it is recommended to follow Gronthos procedure.¹⁰⁰

4.4 | Hair follicle

The most difficult step is separation of the follicle from hair. One way is using skin specimens. Skin biopsies can be obtained from different parts of the body, namely from the face, back, forearm, thigh, calf, chest and pubic region. As Ohyama et al.⁸ stated, microdissecting approaches have been the standard method for obtaining HF; however, other researchers provided various protocols; Krejci et al.¹⁰¹ excised an area under the sebaceous gland because bulge was not a clearly visible structure during preparation. Clewes et al. separated the bulge region completely from other parts by removing the dermal papilla and matrix. They also stated that, for protection against oxidative stress, low oxygen tension was applied for culture condition. They noted that hypoxia is defined as O_2 pressure below the normoxic value in a given tissue and in HFs; oxygen tension ranges between 2.5% and 0.1% O_2 .¹⁰² Magerl et al. recommended a simplified protocol for isolation of HF; they transected small skin specimens (approximately 1 cm³) at the dermis-subcutis interface by scalpel to expose the mid-to-lower segments of HF embedded in the AD. The upper portion of the HFs from the subcutis was ejected by pressing the sides of the fat tissue with blunt forceps.¹⁰³ The simplest method was presented by Wang et al. They plucked hair with a full HF from scalp, and simply cut the root tissue with sterile ophthalmic scissors and used the root for explantation.¹⁰⁴

4.5 | Cornea

Different populations of stem cells have been detected in human corneal tissue.¹⁰⁵ Recently, MSC was also isolated from human cornea

and found to play role in injury/wound healing process within the tissue.^{9,106} Luznic et al. explanted limbal rim pieces including the epithelium as well as some of the superficial limbal stromal tissue. They detected two distinct population of stem cells after long-term culture (when explants were cultivated for over 30 days) and found that after a second 30-day explantation of same pieces into another dish, MSC population became 2-fold in number.¹⁰⁶ In another study, Vereb et al. isolated MSCs from just central part of the cornea; by their method, just MSC population proliferated and detected in the culture with no endothelial cell. Their isolation method and culturing condition led to obtaining cells with CD90- and CD105-positive and CD34-negative markers over 10 passages.⁹

4.6 | Umbilical cord

Mesenchymal stem cells can be isolated from either whole UC¹⁰⁷ or Wharton's jelly matrix.¹⁰⁸ Although isolated cells from both showed similar properties, the methods are somehow different, especially in considering large-scale set-up. Cell isolation from Wharton's jelly is more labour-intensive than from entire cord piece, because Wharton's jelly must be separated from other parts—umbilical vein and arteries—which is a time-consuming and labour-intensive process. On the other hand, in isolation of cells from entire cord piece, there is no need to separate different parts of the cord, and by simple and rapid incisions for better tissue exposure, the tissue can be explanted for primary culture.¹⁰⁹

For isolation of MSCs from human UC, Mori et al. used a stainless steel mesh (Cellamigo[®]; Tsubakimoto Chain Co. Saitama, Japan.) to prevent tissue explant pieces from floating; they stated that by this improvement, cells can be obtained sooner and with higher yield compared to conventional explant method.¹¹⁰

4.7 | Placenta

Due to high probability of transmission of normal flora after vaginal delivery, it is highly recommended to collect placenta after sterile elective caesarean sections, it is also necessary to confirm donor health with no bloodborne infections especially hepatitis, HIV and syphilis.

It is possible to isolate MSCs from foetal or maternal parts of the placenta.¹¹¹ Amniotic membrane can be isolated from chorion by blunt dissection.¹¹² To isolate MSCs from chorionic villi, maternal deciduas should be separated and discarded, and then chorionic villi should be cut from the foetal portion.^{113,114}

5 | COMPARISON OF MSCS ISOLATED BY ENZYMATIC AND EXPLANT METHODS

In a number of studies, MSCs were isolated by explant and enzymatic methods from the same tissue and compared for main characteristics. Priya et al. measured surface expression of CD markers (CD34, CD44, CD73, CD90, CD105, HLA-DR) on MSCs obtained by explant and enzymatic methods at P0, P1, P4, P10. They showed that there

TABLE 1 Technical details for MSC isolation from human adult and perinatal tissues by explant primary culture method

Study	Specimen	Preparation	Plating
<i>Adipose tissue</i>			
91	10 mL Lipoaspirate 5 g resected adipose tissue	Fragments of about 5 mm ³	1 g tissue/100 mm dish
92	Small pieces of rat adipose tissue Human lipoaspirate	Small pieces (≈5 mg)	Explanting tissue pieces into the corner of flask
93	Inguinal fat pads from 8-week-old BALB/c mice	1-2 mm ³ pieces	Spacing between adjacent tissues = 5 mm 5 min for tissue adherence
94	Lipoaspirate	Saline/blood fraction located below the floating, more buoyant adipose tissue	Resuspending the cell pellet in DMEM with 40-50% FBS followed by plating the cells
<i>Bone marrow</i>			
95	Bone marrow sample	Dilution with three equal volumes of growth medium	10 mL of diluted aspirate in each 75-80 cm ² flask or 10-cm dish
5	Bone marrow sample	Centrifuging cell suspension (1000 rpm, 5 min, at 18°C), resuspending cell pellet in complete medium	Seeding the cell suspension in 25 cm ² flasks (16 × 10 ⁶ cells/mL)
<i>Dental pulp</i>			
97	Extracted third or premolar teeth	Removing pulp tissue from tooth and cutting into small pieces	Explanting pieces on culture surface and covering them by plastic coverslips
96	Third molar teeth	Gentle removal of pulp and mincing into 1-2 mm ³ fragments	Placing pieces in six well plates
<i>Hair follicle</i>			
104	Plucked hairs with full hair follicle	Cutting the root tissue with sterile ophthalmic scissors	Placing hair follicle root tissues into 96-well plates
<i>Cornea</i>			
9	Corneal buttons from cadavers	Scrapping epithelium, the Bowman's membrane, then endothelium and the Descemet's membrane, to obtain just central part of cornea and cutting it into small squares	Plating pieces into 24-well cell culture plates
106	Limbal tissue	Incision and separation of conjunctiva from the limbal junction. Cutting limbal rim into ~1 × 2 × 0.25 mm pieces	Placing each limbal explant with the epithelium side down into a well of 12 well plates
<i>Umbilical cord/Wharton's jelly</i>			
109	Umbilical cord	Cutting UC in to 1.5 cm length pieces	Plating one piece/6 cm dish, 5-10 min for tissue attachment
109	Umbilical cord	Excising the gelatinous tissue surrounding the vessels and mincing into very fine pieces of 1-3 mm ²	Plating 10-14 piece/6 cm plate, 5-10 min for tissue attachment
110	Umbilical cord	Mincing UCs into 1-2-mm ³ fragments	Aligning 2 g minced UC at regular intervals in 10-cm culture dishes and covering them with Cellamigo® mesh
<i>Placenta</i>			
113	Placenta	Separating maternal deciduas and cutting chorionic villi from foetal portion into 40 mg pieces (wet weight)	Allowing the tissues to adhere to the plastic surface in six-well plates for 60 min
114	Placenta	Removing amnion and chorionic plate from placenta and cutting foetal villi into small pieces	Placing tissue pieces into dishes with no coating to attach to the surface

NM, not mentioned.

First medium	First medium change	Explant removal	First observation of migrated cells	Yield
2.5 mL knock-out DMEM + 10% FBS + 2 mmol/L-glutamine + antibiotics	Not directly mentioned (it seems to be 5-7 d after plating)	5-7 d after plating	3-4 d after plating	$5-8 \times 10^5$ cells/g of initial tissue plated (10-14 d after plating)
50 μ L pre-warmed FBS	After 24 h DMEM + antibiotic + 20% FBS	3 d after plating	3-5 d after seeding	NM
α -MEM + 10% FBS	2 d after plating	NM	2 d after plating	24×10^3 cells/mg adipose tissue after 7 d of culture
DMEM + 40%-50% FBS	After an overnight incubation DMEM low glucose + 10% FBS + 1% ABAM+ $\pm$ 10 ng/mL EGF	After an overnight incubation	NM	$\sim$ 100 000 cells/100 mL of blood/saline collected
DMEM low glucose + 10% FBS + antibiotics	Undisturbed at 37°C for 4-5 d	After 4-5 d	Small MSC colonies are visible at 5-7 d	NM
RPMI-1640 with 10% FBS and antibiotics	After 6 and 24 h	After 6 and 24 h	Attached cells after 6 h	NM
DMEM + 2 mmol/L-glutamine + antibiotics + 20% FBS	After 5 d	NM	NM	NM
α -MEM + 2 mmol/L α -glutamine + antibiotics + 10% FBS	NM	NM	2-4 d	NM
DMEM/Ham's DMEM/F12 + 10% FBS + 2 ng/mL bFGF	NM	NM	NM	NM
1 mL low-glucose DMEM + 10% FCS + 1% antibiotic- antimycotic solution	Every alternate day	NM	After 10-14 d	NM
1:1 mixture of DMEM and Ham's F12 medium + 10%/20% human serum + 50 μ g/mL gentamicin and penicillin	Every 2-3 d	Remained for prolonged time period (60-61 d)	NM	NM
5 mL of high-glucose DMEM + 10% FBS + 2 mmol/L α -glutamine + antibiotics	4 d post-explantation	Once floating by itself	24 h after plating	NM
5 mL of high-glucose DMEM supplemented with 10% FBS, 2 mmol/L α -glutamine, and antibiotics	4 d post-explantation	Once floating by itself	24 h after plating	NM
α -MEM supplemented with 10% FBS, gentamicin, and amphotericin B	5 d after plating	Within 9-12 d	NM	$2.9 \pm 1.4 \times 10^6$ cells/g
DMEM-F12 + 10% FBS + 100 μ g/mL α -glutamate + antibiotics	2 d after plating	14 d after explantation	NM	11.55×10^3 cells/40 mg tissue
DMEM (low glucose) + 10% FBS + antibiotics/antimycotics	NM	NM	NM	1×10^4 cells/piece after 20 d

were just little differences between MSCs which were isolated by two methods. They also found no difference between immunogenic and immunosuppressive activities of explant and enzymatic-derived MSC; MSCs did not stimulate proliferation of peripheral blood mononuclear cells (PBMCs), and also strongly suppressed lymphocyte proliferation after mixed lymphocyte (MLR) reaction.⁹¹ Explant method led to harvest pure and less heterogeneous cells with higher proliferation rates when compared with enzymatic method^{13,96}; enzymatic digestion allowed the isolation of fibroblast-like (stem) cells and also release of endothelial cells and pericytes.⁹⁶ Salehinejad et al.¹³ suggested that more homogenous cell population and less enzymatic damages was the reason for higher proliferation rate of MSCs derived by explant method. Significantly lower percentage of HLA-DR⁺ hematopoietic cells in passage 0 of cells harvested by explant method was again reported by Priya et al.,⁹¹ which indicated more homogenous MSC population. Yoon et al.¹⁴ also isolated MSCs from Wharton's jelly by explant and enzymatic methods and stated that explant method yielded in higher cell viability and number (2.8 times greater number of cells than enzymatic digestion-derived MSCs at P0). Jing et al. obtained few cells in the first 2 days post-explantation; however, the cell number increased very fast in the next days and at last the primary cultured cells obtained by explant and enzymatic methods grew to 80% confluence approximately at the same time. They calculated the nucleated cell number per tissue weight and showed that the explant culture method gave higher yield of stromal cells than the digestion method; may be due to the decreased adhesion ability in the cells exposed to enzymatic treatment, and the loss of cells during the procedures of filtering and washing in the digestion method.⁹³ Shah et al. isolated MSCs from human lipoaspirates by two methods; in the first, they used collagenase and in the second, applied washing steps without any protease. Tissue processing time in non-enzymatic method was one-third of the time it took in enzymatic method, and time to

reach confluence in enzyme method was less than half of the time for washing method. While primary cell yield was about 19-fold greater, there was no significant difference according to adipogenic and osteogenic differentiations, and in passage 0, MSCs from both methods showed comparable immunophenotype.¹¹⁵ Jing et al.⁹³ also agreed in shorter processing time in explant culture compared to digestion method; as it took 1 hour to disaggregate the tissue by collagenase. In this regard, Ghorbani et al. pointed that their explant procedure would be a suitable method for isolation of MSCs from small amounts of fat tissue and applicable for patients suffering from cancer and some chronic diseases which are associated with pronounced fat lost (cachexia) and a limited amount of fat can be obtained⁹² (Figure 4).

Cryopreservation is currently the best way for long-term cell storage; MSCs, isolated by explant method, can be successfully revitalized after cryopreservation while maintaining their characteristics.^{109,116} In addition, after cryopreservation of UC tissue, it is possible to isolate MSCs by explant method from post-thaw tissue pieces.^{117,118}

6 | RESEARCH IN PROGRESS AND FUTURE DIRECTIONS

One of the most important challenges in preparation of cell therapy products is the application of xenogenic biologicals such as proteolytic enzymes and serum. In MSC isolation and culture, proteolytic enzymes are used during two steps: isolation and subculturing. In explant method, enzymes are omitted from isolation step; however, they are still necessary for passaging. 3D culture systems can be helpful for MSC propagation without application of proteolytic enzymes; however, these systems are still improving.¹¹⁹

For removing animal serum from MSC culture, it is suggested to use human serum obtained from the donor, volunteers or UC blood.¹²⁰

FIGURE 4 Comparison of main characteristics of explant-derived MSCs with those obtained after enzymatic digestion of the original tissues. MSCs which were obtained using explant method were successfully differentiated into mesodermal lineages (osteocytes, adipocytes and chondrocytes),^{13,91,96} they also express stromal markers (CD29, CD44, CD73, CD90, CD45, HLA-DR) in a comparable manner to enzymatic-derived MSCs⁹⁶; in some cases such as CD44, CD73 and CD90 expression, their level were higher in explant-derived MSCs, while CD34 and CD45 expression level were lower.^{13,115} They showed similar morphology, immunogenicity and immunosuppressive properties. Similar cell yield and doubling time and proliferation rate was also reported for explant-derived MSCs^{13,91,92}

Human platelet lysate is also recently recommended to be used instead of animal serum.^{116,121}

Another issue is insufficient cell numbers which was mostly of concern in the case of BM as the most common MSC source. Going towards use of medical waste tissues such as UC and AD, that are available in large amounts, can resolve this problem completely.

Until now explant culture method was applied for isolation of MSCs from few tissue types (which are presented in the current review) as main MSC sources; however, it is highly suggested to apply it for MSC isolation from any other tissue source.

7 | CONCLUSION

Mesenchymal stem cell isolation from human tissues can be performed efficiently using explant culture method. Obtained MSCs possess similar characteristics with those derived by enzyme digestion. However, explant method includes several advantages comparing with enzymatic isolation. These advantages are of significant importance because in explant isolation, proteolytic stress on cells is omitted and they are also in companion with a piece of their tissue origin in the primary culture which increase probability of successful and high yield isolation. Reduced price and risk of biological contaminations are other influential advantages (regarding economic and labour issue). They are satisfactory reasons for using explant methods for MSC isolation in research and clinical settings, against longer time for obtaining cells in primary culture initial step comparing to enzymatic method. Further investigations are recommended to modify explant methods to make this lag time shorter. More experiments can also help us to even better understand the molecular events in primary culture in order to optimize and design isolation step more precisely.

CONFLICT OF INTEREST

There is no conflict of interest. There is no grant or funding support for this manuscript.

REFERENCES

- Hendijani F, Javanmard SH. Dual protective and cytotoxic benefits of mesenchymal stem cell therapy in combination with chemotherapy/radiotherapy for cancer patients. *Crit Rev Eukaryot Gene Expr*. 2015;25:203–207.
- Hendijani F, Javanmard SH, Sadeghi-aliabadi H. Human Wharton's jelly mesenchymal stem cell secretome display antiproliferative effect on leukemia cell line and produce additive cytotoxic effect in combination with doxorubicin. *Tissue Cell*. 2015;47:229–234.
- Hendijani F, Javanmard SH, Rafiee L, Sadeghi-Aliabadi H. Effect of human Wharton's jelly mesenchymal stem cell secretome on proliferation, apoptosis and drug resistance of lung cancer cells. *Res Pharm Sci*. 2015;10:134–142.
- Lalu MM, McIntyre L, Pugliese C, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS ONE*. 2012;7:e47559.
- AL-Qaisy BA, Yousif Yaseen N, Alwachi SN, AL-Shammari AM. Comparison between three different protocols for isolation and culture of mouse bone marrow derived mesenchymal stem cells. *Iraqi J Cancer Med Genet*. 2014;7:26–35.
- Eom YW, Lee JE, Yang MS, et al. Rapid isolation of adipose tissue-derived stem cells by the storage of lipoaspirates. *Yonsei Med J*. 2011;52:999–1007.
- Chen YK, Huang AH, Chan AW, Shieh TY, Lin LM. Human dental pulp stem cells derived from different cryopreservation methods of human dental pulp tissues of diseased teeth. *J Oral Pathol Med*. 2011;40:793–800.
- Ohya M, Zheng Y, Paus R, Stenn KS. The mesenchymal component of hair follicle neogenesis: background, methods and molecular characterization. *Exp Dermatol*. 2010;19:89–99.
- Vereb Z, Poliska S, Albert R, et al. Role of human corneal stroma-derived mesenchymal-like stem cells in corneal immunity and wound healing. *Sci Rep*. 2016;6:26227.
- Can A, Balci D. Isolation, culture, and characterization of human umbilical cord stroma-derived mesenchymal stem cells [Research Support, Non-U.S. Gov't] 2011. <http://www.ncbi.nlm.nih.gov/pubmed/21431510>. Last accessed date: 17 October 2017
- Hass R, Kasper C, Bohm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. *Cell Commun Signal*. 2011;9:12.
- Freshney RI. *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications*, 6th ed. Hoboken, NJ: Wiley-Blackwell; 2010. xxxi, 732 p., 28 p. of plates p.
- Salehinejad P, Banu Alitheen N, Ali AM, et al. Comparison of different methods for the isolation of mesenchymal stem cells from human umbilical cord Wharton's jelly. *In Vitro Cell Dev Biol Anim*. 2012;48:75–83.
- Yoon JH, Roh EY, Shin S, et al. Comparison of explant-derived and enzymatic digestion-derived MSCs and the growth factors from Wharton's jelly. *BioMed Res Int*. 2013;2013:428726.
- Humphries JD, Byron A, Humphries MJ. Integrin ligands at a glance. *J Cell Sci*. 2006;119:3901–3903.
- Hynes RO. The extracellular matrix: not just pretty fibrils. *Science*. 2009;326:1216–1219.
- Xian X, Gopal S, Couchman JR. Syndecans as receptors and organizers of the extracellular matrix. *Cell Tissue Res*. 2010;339:31–46.
- Schmidt S, Friedl P. Interstitial cell migration: integrin-dependent and alternative adhesion mechanisms. *Cell Tissue Res*. 2010;339:83–92.
- Iyer AK, Tran KT, Borysenko CW, et al. Tenascin cytotactin epidermal growth factor-like repeat binds epidermal growth factor receptor with low affinity. *J Cell Physiol*. 2007;211:748–758.
- Panayotou G, End P, Aumailley M, Timpl R, Engel J. Domains of laminin with growth-factor activity. *Cell*. 1989;56:93–101.
- Wang X, Harris RE, Bayston LJ, Ashe HL. Type IV collagens regulate BMP signalling in Drosophila. *Nature*. 2008;455:72–77.
- Brizzi MF, Tarone G, Defilippi P. Extracellular matrix, integrins, and growth factors as tailors of the stem cell niche. *Curr Opin Cell Biol*. 2012;24:645–651.
- Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. *J Cell Sci*. 2010;123:4195–4200.
- Fuchs E, Tumber T, Guasch G. Socializing with the neighbors: stem cells and their niche. *Cell*. 2004;116:769–778.
- Watt FM, Fujiwara H. Cell-extracellular matrix interactions in normal and diseased skin. *Cold Spring Harb Perspect Biol*. 2011;3:a005124. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3062212/pdf/csh-perspect-ECM-a005124.pdf>
- Kurtz A, Oh SJ. Age related changes of the extracellular matrix and stem cell maintenance. *Prev Med*. 2012;54(suppl):S50–S56.
- Korpos E, Wu C, Sorokin L. Multiple roles of the extracellular matrix in inflammation. *Curr Pharm Des*. 2009;15:1349–1357.
- Pera MF, Tam PP. Extrinsic regulation of pluripotent stem cells. *Nature*. 2010;465:713–720.

29. Clark RA. Fibrin and wound healing. *Ann N Y Acad Sci.* 2001;936:355–367.
30. Schultz GS, Wysocki A. Interactions between extracellular matrix and growth factors in wound healing. *Wound Repair Regen.* 2009;17:153–162.
31. Schafer M, Werner S. Cancer as an overhealing wound: an old hypothesis revisited. *Nat Rev Mol Cell Biol.* 2008;9:628–638.
32. Fong CY, Subramanian A, Biswas A, et al. Derivation efficiency, cell proliferation, freeze-thaw survival, stem-cell properties and differentiation of human Wharton's jelly stem cells. *Reprod Biomed Online.* 2010;21:391–401.
33. Kang YJ, Jeon ES, Song HY, et al. Role of c-Jun N-terminal kinase in the PDGF-induced proliferation and migration of human adipose tissue-derived mesenchymal stem cells. *J Cell Biochem.* 2005;95:1135–1145.
34. Ng F, Boucher S, Koh S, et al. PDGF, TGF-beta, and FGF signaling is important for differentiation and growth of mesenchymal stem cells (MSCs): transcriptional profiling can identify markers and signaling pathways important in differentiation of MSCs into adipogenic, chondrogenic, and osteogenic lineages. *Blood.* 2008;112:295–307.
35. Stewart AA, Byron CR, Pondenis H, Stewart MC. Effect of fibroblast growth factor-2 on equine mesenchymal stem cell monolayer expansion and chondrogenesis. *Am J Vet Res.* 2007;68:941–945.
36. Tong CK, Vellamy S, Tan BC, et al. Generation of mesenchymal stem cell from human umbilical cord tissue using a combination enzymatic and mechanical disassociation method. *Cell Biol Int.* 2011;35:221–226.
37. Tsutsumi S, Shimazu A, Miyazaki K, et al. Retention of multilineage differentiation potential of mesenchymal cells during proliferation in response to FGF. *Biochem Biophys Res Commun.* 2001;288:413–419.
38. Miyazawa-Hoshimoto S, Takahashi K, Bujo H, Hashimoto N, Yagui K, Saito Y. Roles of degree of fat deposition and its localization on VEGF expression in adipocytes. *Am J Physiol Endocrinol Metab.* 2005;288:E1128–E1136.
39. Sung HK, Doh KO, Son JE, et al. Adipose vascular endothelial growth factor regulates metabolic homeostasis through angiogenesis. *Cell Metab.* 2013;17:61–72.
40. Mick GJ, Wang X, McCormick K. White adipocyte vascular endothelial growth factor: regulation by insulin. *Endocrinology.* 2002;143:948–953.
41. Zhang QX, Magovern CJ, Mack CA, Budenbender KT, Ko W, Rosengart TK. Vascular endothelial growth factor is the major angiogenic factor in omentum: mechanism of the omentum-mediated angiogenesis. *J Surg Res.* 1997;67:147–154.
42. Gabrielsson BG, Johansson JM, Jennische E, et al. Depot-specific expression of fibroblast growth factors in human adipose tissue. *Obes Res.* 2002;10:608–616.
43. Chen X, Li Y, Wang L, et al. Ischemic rat brain extracts induce human marrow stromal cell growth factor production. *Neuropathology.* 2002;22:275–279.
44. Smiler DG, Soltan M, Soltan C, Matthews C. Growth factors and gene expression of stem cells: bone marrow compared with peripheral blood. *Implant dentistry.* 2010;19:229–240.
45. Korf-Klingebiel M, Kempf T, Sauer T, et al. Bone marrow cells are a rich source of growth factors and cytokines: implications for cell therapy trials after myocardial infarction. *Eur Heart J.* 2008;29:2851–2858.
46. Takai K, Hara J, Matsumoto K, et al. Hepatocyte growth factor is constitutively produced by human bone marrow stromal cells and indirectly promotes hematopoiesis. *Blood.* 1997;89:1560–1565.
47. Hendijani F (2015) Human mesenchymal stromal cell therapy for prevention and recovery of chemo/radiotherapy adverse reactions. *Cytotherapy.* 2015;17:509–525. doi:10.1016/j.jcyt.2014.10.015
48. Chen X, Wang ZY, Liu SJ. Expression of basic fibroblast growth factor in dental pulp of immature permanent teeth. *Shanghai kou qiang yi xue = Shanghai J Stomatol.* 2003;12:41–43.
49. Derringer KA, Linden RW. Vascular endothelial growth factor, fibroblast growth factor 2, platelet derived growth factor and transforming growth factor beta released in human dental pulp following orthodontic force. *Arch Oral Biol.* 2004;49:631–641.
50. El Karim IA, Linden GJ, Irwin CR, Lundy FT. Neuropeptides regulate expression of angiogenic growth factors in human dental pulp fibroblasts. *J Endod.* 2009;35:829–833.
51. Roberts-Clark DJ, Smith AJ. Angiogenic growth factors in human dentine matrix. *Arch Oral Biol.* 2000;45:1013–1016.
52. Sloan AJ, Perry H, Matthews JB, Smith AJ. Transforming growth factor-beta isoform expression in mature human healthy and carious molar teeth. *Histochem J.* 2000;32:247–252.
53. Smith AJ. Vitality of the dentin-pulp complex in health and disease: growth factors as key mediators. *J Dent Educ.* 2003;67:678–689.
54. Alonso L, Fuchs E. The hair cycle. *J Cell Sci.* 2006;119:391–393.
55. du Cros DL. Fibroblast growth factor and the hair cycle of the hairless mouse. *J Invest Dermatol.* 1995;104:175–185.
56. Werner S, Smola H, Liao X, et al. The function of KGF in morphogenesis of epithelium and reepithelialization of wounds. *Science.* 1994;266:819–822.
57. Widelitz RB, Jiang TX, Noveen A, Chen CW, Chuong CM. FGF induces new feather buds from developing avian skin. *J Invest Dermatol.* 1996;107:797–803.
58. Kozłowska U, Blume-Peytavi U, Kodellja V, et al. Expression of vascular endothelial growth factor (VEGF) in various compartments of the human hair follicle. *Arch Dermatol Res.* 1998;290:661–668.
59. Lindner G, Menrad A, Gherardi E, et al. Involvement of hepatocyte growth factor/scatter factor and met receptor signaling in hair follicle morphogenesis and cycling. *FASEB J.* 2000;14:319–332.
60. Akiyama M, Smith LT, Holbrook KA. Growth factor and growth factor receptor localization in the hair follicle bulge and associated tissue in human fetus. *J Invest Dermatol.* 1996;106:391–396.
61. Kawano M, Komi-Kuramochi A, Asada M, et al. Comprehensive analysis of FGF and FGFR expression in skin: FGF18 is highly expressed in hair follicles and capable of inducing anagen from telogen stage hair follicles. *J Invest Dermatol.* 2005;124:877–885.
62. Kawano M, Suzuki S, Suzuki M, Oki J, Imamura T. Bulge- and basal layer-specific expression of fibroblast growth factor-13 (FHF-2) in mouse skin. *J Invest Dermatol.* 2004;122:1084–1090.
63. Moore GP, Du Cros DL, Isaacs K, Pisansarakit P, Wynn PC. Hair growth induction: roles of growth factors. *Ann N Y Acad Sci.* 1991;642:308–325.
64. Klenkler B, Sheardown H. Growth factors in the anterior segment: role in tissue maintenance, wound healing and ocular pathology. *Exp Eye Res.* 2004;79:677–688.
65. Secker GA, Daniels JT. *Limbal Epithelial Stem Cells of the Cornea.* Cambridge, MA: StemBook; 2008.
66. Wilson SE, Chen L, Mohan RR, Liang Q, Liu J. Expression of HGF, KGF, EGF and receptor messenger RNAs following corneal epithelial wounding. *Exp Eye Res.* 1999;68:377–397.
67. Imanishi J, Kamiyama K, Iguchi I, Kita M, Sotozono C, Kinoshita S. Growth factors: importance in wound healing and maintenance of transparency of the cornea. *Prog Retin Eye Res.* 2000;19:113–129.
68. Wilson SE, Schultz GS, Chegini N, Weng J, He YG. Epidermal growth factor, transforming growth factor alpha, transforming growth factor beta, acidic fibroblast growth factor, basic fibroblast growth factor, and interleukin-1 proteins in the cornea. *Exp Eye Res.* 1994;59:63–71.
69. Yu FS, Yin J, Xu K, Huang J. Growth factors and corneal epithelial wound healing. *Brain Res Bull.* 2010;81:229–235.
70. Sobolewski K, Malkowski A, Bankowski E, Jaworski S. Wharton's jelly as a reservoir of peptide growth factors. *Placenta.* 2005;26:747–752.
71. Ahmed A, Li XF, Dunk C, Whittle MJ, Rushton DI, Rollason T. Colocalisation of vascular endothelial growth factor and its Flt-1 receptor in human placenta. *Growth Factors.* 1995;12:235–243.

72. Sharkey AM, Charnock-Jones DS, Boocock CA, Brown KD, Smith SK. Expression of mRNA for vascular endothelial growth factor in human placenta. *J Reprod Fertil*. 1993;99:609–615.
73. Clark DE, Smith SK, Licence D, Evans AL, Charnock-Jones DS. Comparison of expression patterns for placenta growth factor, vascular endothelial growth factor (VEGF), VEGF-B and VEGF-C in the human placenta throughout gestation. *J Endocrinol*. 1998;159:459–467.
74. Arany E, Hill DJ. Fibroblast growth factor-2 and fibroblast growth factor receptor-1 mRNA expression and peptide localization in placentae from normal and diabetic pregnancies. *Placenta*. 1998;19:133–142.
75. Shams M, Ahmed A. Localization of mRNA for basic fibroblast growth factor in human placenta. *Growth Factors*. 1994;11:105–111.
76. Ferretti C, Bruni L, Dangles-Marie V, Pecking AP, Bellet D. Molecular circuits shared by placental and cancer cells, and their implications in the proliferative, invasive and migratory capacities of trophoblasts. *Hum Reprod Update*. 2007;13:121–141.
77. Goustin AS, Betsholtz C, Pfeifer-Ohlsson S, et al. Coexpression of the sis and myc proto-oncogenes in developing human placenta suggests autocrine control of trophoblast growth. *Cell*. 1985;41:301–312.
78. Jurcovicova J, Krueger KS, Nandy I, Lewis DF, Brooks GG, Brown EG. Expression of platelet-derived growth factor-A mRNA in human placenta: effect of magnesium infusion in pre-eclampsia. *Placenta*. 1998;19:423–427.
79. Kauma S, Hayes N, Weatherford S. The differential expression of hepatocyte growth factor and met in human placenta. *J Clin Endocrinol Metab*. 1997;82:949–954.
80. Ladines-Llave CA, Maruo T, Manalo AS, Mochizuki M. Cytologic localization of epidermal growth factor and its receptor in developing human placenta varies over the course of pregnancy. *Am J Obstet Gynecol*. 1991;165:1377–1382.
81. Maruo T, Murata K, Matsuo H, Samoto T, Mochizuki M. Insulin-like growth factor-I as a local regulator of proliferation and differentiated function of the human trophoblast in early pregnancy. *Early Pregnancy*. 1995;1:54–61.
82. Ohlsson R, Holmgren L, Glaser A, Szepecht A, Pfeifer-Ohlsson S. Insulin-like growth factor 2 and short-range stimulatory loops in control of human placental growth. *The EMBO J*. 1989;8:1993–1999.
83. Sutradhar BC, Park J, Hong G, Choi SH, Kim G. Effects of trypsinization on viability of equine chondrocytes in cell culture. *Pak Vet J*. 2010;30:232–238.
84. Fakhri O, Tan RS. Short communications. The effect of trypsin on cell surface antigens. *Cell Immunol*. 1975;15:452–456.
85. Furcht LT, Wendelschafer-Crabb G. Trypsin-induced coordinate alterations in cell shape, cytoskeleton, and intrinsic membrane structure of contact-inhibited cells. *Exp Cell Res*. 1978;114:1–14.
86. Kellner G, Broda E, Suschny O, Rucker W. Effects of trypsin treatment on tissue in culture. *Exp Cell Res*. 1959;18:168–171.
87. Atala A, Lanza RP. *Methods of Tissue Engineering*. San Diego: Academic Press; 2002.
88. Otte A, Bucan V, Reimers K, Hass R. Mesenchymal stem cells maintain long-term in vitro stemness during explant culture. *Tissue Eng Part C Methods*. 2013;19:937–948.
89. Marcus-Sekura C, Richardson JC, Harston RK, Sane N, Sheets RL. Evaluation of the human host range of bovine and porcine viruses that may contaminate bovine serum and porcine trypsin used in the manufacture of biological products. *Biologicals*. 2011;39:359–369.
90. Pinheiro de Oliveira TF, Fonseca AA Jr, Camargos MF, et al. Detection of contaminants in cell cultures, sera and trypsin. *Biologicals*. 2013;41:407–414.
91. Priya N, Sarcar S, Majumdar AS, SundarRaj S. Explant culture: a simple, reproducible, efficient and economic technique for isolation of mesenchymal stromal cells from human adipose tissue and lipoaspirate. *J Tissue Eng Regen Med*. 2014;8:706–716.
92. Ghorbani A, Jalali SA, Varedi M. Isolation of adipose tissue mesenchymal stem cells without tissue destruction: a non-enzymatic method. *Tissue Cell*. 2014;46:54–58.
93. Jing W, Xiao J, Xiong Z, et al. Explant culture: an efficient method to isolate adipose-derived stromal cells for tissue engineering. *Artif Organs*. 2011;35:105–112.
94. Francis MP, Sachs PC, Elmore LW, Holt SE. Isolating adipose-derived mesenchymal stem cells from lipoaspirate blood and saline fraction. *Organogenesis*. 2010;6:11–14.
95. Pittenger MF. Mesenchymal stem cells from adult bone marrow. *Methods Mol Biol*. 2008;449:27–44.
96. Hilken P, Gervois P, Fanton Y, et al. Effect of isolation methodology on stem cell properties and multilineage differentiation potential of human dental pulp stem cells. *Cell Tissue Res*. 2013;353:65–78.
97. Jiang L, Peng WW, Li LF, Yang Y, Zhu YQ. Isolation and identification of CXCR4-positive cells from human dental pulp cells. *J Endod*. 2012;38:791–795.
98. Laino G, d'Aquino R, Graziano A, et al. A new population of human adult dental pulp stem cells: a useful source of living autologous fibrous bone tissue (LAB). *Journal Bone Miner Res*. 2005;20:1394–1402.
99. Perry BC, Zhou D, Wu X, et al. Collection, cryopreservation, and characterization of human dental pulp-derived mesenchymal stem cells for banking and clinical use. *Tissue Eng Part C Methods*. 2008;14:149–156.
100. Gronthos S, Arthur A, Bartold PM, Shi S. A method to isolate and culture expand human dental pulp stem cells. *Methods Mol Biol*. 2011;698:107–121.
101. Krejci E, Grim M. Isolation and characterization of neural crest stem cells from adult human hair follicles. *Folia Biol*. 2010;56:149–157.
102. Clewes O, Narytnyk A, Gillinder KR, Loughney AD, Murdoch AP, Sieber-Blum M. Human epidermal neural crest stem cells (hEPI-NCSC)—characterization and directed differentiation into osteocytes and melanocytes. *Stem Cell Rev*. 2011;7:799–814.
103. Magerl M, Kauser S, Paus R, Tobin DJ. Simple and rapid method to isolate and culture follicular papillae from human scalp hair follicles. *Exp Dermatol*. 2002;11:381–385.
104. Wang Y, Liu J, Tan X, et al. Induced pluripotent stem cells from human hair follicle mesenchymal stem cells. *Stem Cell Rev*. 2013;9:451–460.
105. Funderburgh JL, Funderburgh ML, Du Y. Stem cells in the limbal stroma. *Ocul Surf*. 2016;14:113–120.
106. Luznik Z, Hawlina M, Malicev E, et al. Effect of cryopreserved amniotic membrane orientation on the expression of limbal mesenchymal and epithelial stem cell markers in prolonged limbal explant cultures. *PLoS ONE*. 2016;11:e0164408.
107. La Rocca G, Anzalone R, Corrao S, et al. Isolation and characterization of Oct-4+/HLA-G+ mesenchymal stem cells from human umbilical cord matrix: differentiation potential and detection of new markers. *Histochem Cell Biol*. 2009;131:267–282.
108. Petsa A, Gargani S, Felesakis A, Grigoriadis N, Grigoriadis I. Effectiveness of protocol for the isolation of Wharton's Jelly stem cells in large-scale applications. *In Vitro Cell Dev Biol Anim*. 2009;45:573–576.
109. Hendijani F, Sadeghi-Aliabadi H, Haghjooy Javanmard S. Comparison of human mesenchymal stem cells isolated by explant culture method from entire umbilical cord and Wharton's jelly matrix. *Cell Tissue Banking*. 2014;15:555–565.
110. Mori Y, Ohshimo J, Shimazu T, et al. Improved explant method to isolate umbilical cord-derived mesenchymal stem cells and their immunosuppressive properties. *Tissue Eng Part C Methods*. 2015;21:367–372.
111. In't Anker PS, Scherjon SA, Kleijburg-van der Keur C, et al. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. *Stem Cells*. 2004;22:1338–1345.
112. Tseng SCG. Grafts made from amniotic membrane; methods of separating, preserving, and using such grafts in surgeries. 2000.

113. Abumaree MH, Al Jumah MA, Kalionis B, et al. Phenotypic and functional characterization of mesenchymal stem cells from chorionic villi of human term placenta. *Stem Cell Rev.* 2013;9:16–31.
114. Zhang X, Mitsuru A, Igura K, et al. Mesenchymal progenitor cells derived from chorionic villi of human placenta for cartilage tissue engineering. *Biochem Biophys Res Commun.* 2006;340:944–952.
115. Shah FS, Wu X, Dietrich M, Rood J, Gimble JM. A non-enzymatic method for isolating human adipose tissue-derived stromal stem cells. *Cytotherapy.* 2013;15:979–985.
116. Escobar CH, Chaparro O. Xeno-free extraction, culture, and cryopreservation of human adipose-derived mesenchymal stem cells. *Stem Cells Transl Med.* 2016;5:358–365.
117. Dulugiac M, Moldovan L, Zarnescu O. Comparative studies of mesenchymal stem cells derived from different cord tissue compartments – the influence of cryopreservation and growth media. *Placenta.* 2015;36:1192–1203.
118. Marquez-Curtis LA, Janowska-Wieczorek A, McGann LE, Elliott JA. Mesenchymal stromal cells derived from various tissues: Biological, clinical and cryopreservation aspects. *Cryobiology.* 2015;71:181–197.
119. Saleh FA, Frith JE, Lee JA, Genever PG. Three-dimensional in vitro culture techniques for mesenchymal stem cells. *Methods Mol Biol.* 2012;916:31–45.
120. Shetty P, Bharucha K, Tanavde V. Human umbilical cord blood serum can replace fetal bovine serum in the culture of mesenchymal stem cells. *Cell Biol Int.* 2007;31:293–298.
121. Bieback K. Platelet lysate as replacement for fetal bovine serum in mesenchymal stromal cell cultures. *Transfus Med Hemother.* 2013;40:326–335.

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