

Wharton's Jelly Mesenchymal Stem Cell: Various Protocols for Isolation and Differentiation of Hepatocyte-Like Cells; Narrative Review

Negar Varaa¹, PhD; Saeed Azandeh², PhD; Zahra Khodabandeh³, PhD; Anneh Mohammad Gharravi⁴, PhD

¹Department of Anatomical Sciences, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran;

²Cellular and Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran;

³Stem Cells Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran;

⁴Stem Cell and Tissue Engineering Research Center, Shahrood University of Medical Sciences, Shahrood, Iran

Correspondence:

Saeed Azandeh, PhD;
Cellular and Molecular Research Center,
Faculty of Medicine, Ahvaz Jundishapur
University of Medical Sciences, Ahvaz,
Postal Code: 61335-189, Iran

Tel/Fax: +98 611-3337370

Email: saeed.azandeh@yahoo.com

Received: 13 December 2017

Revised: 4 February 2018

Accepted: 10 May 2018

What's Known

- Wharton's jelly-Mesenchymal stem cells (WJ-MSCs) might be a suitable candidate for stem cell therapy. They have high proliferation rates, wide multipotency, and hypo-immunogenicity. There are several differentiation methods into hepatocyte-like cells, such as induction by cytokines and growth factors, and differentiation of mesenchymal stem cells (MSCs) in 2- and 3-dimensional matrix.

What's New

- In this review, we introduced the explant method as the most effective isolation protocol for Wharton's Jelly (WJ) as well as summarizing and discussing current hepatocyte differentiation protocol; however, the best and most effective protocol is still unknown.

Abstract

There are several differentiation methods for mesenchymal stem cells (MSCs) into hepatocyte-like cell. Investigators reported various hepatic differentiation protocols such as modifying culturing conditions or using various growth factors/cytokines. In this literature review, we compared different MSCs extraction and isolation protocols from Wharton's jelly (WJ) and explored various MSCs differentiation methods.

Various protocols have been recommended for MSCs isolated from WJ, such as enzymatic, enzymatic-explant, and explant methods. In the explant method, valuable time is wasted, but the cost and biological contaminations are reduced and the number of isolated cells are high. However, other features, such as immune phenotype and multiline-age differentiation capacity, do not differ from other methods. There are also several differentiation methods for hepatocyte-like cell including the induction of MSC by cytokines and growth factors, and the differentiation of MSC in 2- and 3-dimensional matrix (2D and 3D). Among several cytokines, hepatocyte growth factor (HGF) and fibroblast growth factor (FGF) are essential. In the early stage of the differentiation, 2D culture is useful, and in the development stage, 3D culture system with HGF and FGF cytokines are more effective in the process of differentiation. Some studies have used 3D culture system in biocompatible scaffolds, such as alginate, collagen, gelatin, and peptide-Gly-Leu-amide (PGLA).

In conclusion, Wharton's jelly-Mesenchymal stem cells (WJ-MSCs) can be considered as an appropriate source for hepatocyte differentiation. Moreover, we introduced the explant method as the most effective protocol. This review attempted to highlight factors in hepatocyte differentiation, but the most effective protocol is not still unknown.

Please cite this article as: Varaa N, Azandeh S, Khodabandeh Z, Gharravi AM. Wharton's Jelly Mesenchymal Stem Cell: Various Protocols for Isolation and Differentiation of Hepatocyte-Like Cells; Narrative Review. *Iran J Med Sci*.

Keywords • Cell differentiation • Mesenchymal stromal cells • Umbilical cord • Wharton jelly • hepatocytes

Introduction

Chronic liver failure, such as cirrhosis, can be stimulated by viral hepatitis, metabolic diseases, alcohol, drugs, and autoimmune processes. Liver transplantation is the most popular procedure for chronic liver disease.¹ However, there are some problems

such as lack of donor, surgical complications, immunological rejection, and high medical costs. Consequently, stem cell therapies can be a new approach to treat end-stage liver diseases.²

Several studies have tried to find the best stem cell source for hepatocyte transplantation.³ Stem cells are isolated from various sources such as preimplantation embryonic, fetuses, and adult organs. These sources have advantages and disadvantages. Human embryonic stem cells (h-ESCs) are pluripotent, but several problems such as insufficient cell numbers, possible teratoma formation or immune rejection after transplantation can hinder their clinical applications.

MSCs can be extracted from several different sources and are plastic-adherent cells that have the capacity to self-renew. Cells defined by the international society for cellular therapy have a specific surface phenotype and can be differentiated into various lineages including bone, cartilage, and adipose.^{5, 6}

MSCs extracted from the bone marrow and adipose tissue have limitations such as being invasive and having a painful procedure while the high degree of viral infection associated with MSCs removed from the bone marrow may lead to a restriction in their usage.⁷ Also, the obtained MSCs from older individuals is hard, since marrow cavity is filled with yellow fat due to aging process.⁸

Umbilical cord-mesenchymal stem cells (UC-MSCs) with similar immune phenotype and multilineage differentiation have higher expansion potential in comparison with bone marrow MSCs (BM-MSCs) and adipose-derived MSCs (ADMSCs).⁹ Umbilical cords are considered to be medical waste; hence their clinical application in research and cell therapy is of no ethical concern. Furthermore, cells isolated from UCs proliferate rapidly in culture and they have the potential for differentiation.¹⁰ UC-MSCs are capable of suppressing the immune response in vitro, which is similar to BM-MSC properties. Many researchers have investigated MSCs extracted from human UCs tissue, which is an acceptable source.¹¹⁻¹⁶ In some studies, MSCs were isolated from different parts of the umbilical cord, such as WJ matrix, perivascular regions,¹⁷ and sub-amnion membrane with various protocols.¹⁸⁻²¹

It is unclear whether human WJ-MSCs can behave as h-ESCs, human MSCs, or both. They have high proliferation rates, wide multipotency, and hypo-immunogenicity, which do not induce teratomas. Human WJ-MSCs (hWJ-MSCs) express low levels of pluripotent embryonic stem cell markers including POU1, NANOG, SOX2,

and LIN28. That explains why hWJ-MSCs do not produce teratomas. Several cytokines such as IL12A, associated with the induction of apoptosis, were significantly upregulated in hWJ-MSCs. These properties propose that hWJ-MSC is an important applicable stem cell source for cell therapy in allogeneic settings without immune rejection.²²

MSCs can be isolated from WJ by different methods, such as enzymatic, enzymatic-explant, and explants.^{12, 15, 16}

Extraction of MSCs is the first step of working with these cells. Recently, there has been extensive research on the transplantation of differentiated cells into damaged tissues for regenerative medicine.²³

There are several differentiation methods for MSC into hepatocyte-like cell including induction of MSC by cytokines and growth factors, and differentiation of MSC in 2- and 3-dimensional matrix (2D and 3D); however, the advantages are still insufficient in clinical applications. Researchers are investigating to improve the methods of hepatic differentiation of MSCs by modifying culture conditions or adding various growth factors/cytokines. We reviewed the literature and compared different extraction and isolation protocols to extract MSCs from WJ. We also explored various MSCs differentiation methods into hepatocyte-like cells and debated the achievements and prospective development.

Human Umbilical Cord (UC)

Human UC matrix originates from extraembryonic mesoderm. The connective UC tissue is within two arteries and a vein in the amniotic wall.²⁴ Five weeks after fertilization, the UC begins to form and gains an average of 50cm. The different cell populations have been identified in WJ, the sub-endothelium of the umbilical vein, and UC blood. WJ is a matrix of mucous connective tissue composed of fibroblast-like stromal cells, collagen fibers, and proteoglycans, situated between the sub-amnion and the perivascular region. The perivascular region surrounding the umbilical vessels is a source of mesenchymal progenitor cells. The third source of MSCs can be found in the sub-endothelium of the umbilical vein. In addition, UC blood is a rich source of hematopoietic stem/progenitor cells but does not contain mesenchymal progenitor cells.^{25, 26} Furthermore, MSCs harvested from UC blood have low efficiency that exhibit a variety of morphologies and differentiations.²⁷ By investigating the umbilical cord, WJ and the sub-endothelium stem cell revealed that typical features of human MSCs could be differentiated

into chondrocytes and hepatocytes.

It should be noted that the number of isolated cells extracted from UC in the vaginal delivery were more than caesarian section births.²⁸ The efficiency and number of cells isolated from each part of umbilical cord are the same, but there is still controversy between researches.^{13, 28, 29}

MSCs Isolation from WJ

MSCs Isolation by Enzymatic Method The Culture of Total Length of UC

Some studies have described how to obtain MSCs from UC, by using the total length of the cord, which leads to an increase in stem cell yield without time-consuming producers. By using an enzymatic procedure, MSC isolation from the three recognized compartments, the perivascular, and the intra-vascular region within the WJ region, is possible. By different enzymatic isolation methods, higher cell populations can be harvested with less time. Approximately 0.95×10^6 cells can be isolated from the UC by using an enzymatic procedure.^{8, 30} In this procedure, when UC is transferred into the laminar hood in complete sterile condition, the entire length of UC is used. The external surface is examined and engraved with a sterile scalpel. Next, the vessels are removed and the pieces of tissue are incubated in enzymatic cocktail containing enzymes such as hyaluronidase, collagenase, and trypsin for 45 minutes to 2 hours at 37 °C. Then, the digested suspension are collected by gravity in the 50 mL conical tube, diluted and centrifuged. Finally, the pellets are cultured.¹²

Culture of UC Parts

Conventionally, 5-7.5 cm-length pieces of UC from the mid-region are transferred into the sterile medium and cut into smaller 1-1.5-cm-length pieces.¹³

In some procedures, to isolate WJ-MSCs from these small pieces without removing the umbilical blood vessels, the matrix containing the WJ is placed face down in a culture flasks or Petri dishes. The flasks containing an enzymatic solution of collagenase and hyaluronidase, in Dulbecco's Modified Eagle's Medium (DMEM), are incubated at 37 °C in a 5% CO₂ in air atmosphere for 45 min to 2h. This allows WJ loosening and separation from the UC without complete digestion. After the incubation period, the cord pieces are transferred to a new Petri dish or culture flask containing fresh DMEM to remove any remaining enzymes. A pair of forceps is used to gently scrape off the gelatinous WJ from the inner compartments into the fresh

medium. The suspension is passed through a syringe fitted with an 18G needle, centrifuged. Finally, cell pellets are re-suspended in culture media. Studies have revealed that approximately $3-4 \times 10^7$ fresh live WJ-MSCs could be obtained from each piece.³¹

Isolation of MSCs with Explants Method

In this procedure, UC is cut into smaller 2-4 cm length pieces. The obtained small pieces of the cord are washed gently, and, then, at the external surface of each piece, with a horizontal section, the outer sheath of UC and its inner surface containing the WJ are extracted into dishes.¹⁵

In the next step, vein and arteries are exposed and removed away from the inner matrix. Then, the inner matrix of WJ is divided into 3-5 mm pieces with a scalpel and washed again with sterile dissection solution. For explant outgrowth, 6-9 pieces are transferred onto the culture dishes and left undisturbed until the jelly is attached to the dishes. Then, a culture medium such as DMEM is added. The culture dishes are left undisturbed for 3-4 days and maintained at 37 °C in a humidified atmosphere containing 5% CO₂. After the attachment of Wharton jelly, the medium of dishes is changed 2-3 days. Approximately, after 7-10 days, the cells with an MSCs phenotype can be isolated.^{14, 32}

Mori and others used a stainless steel mesh to prevent tissue explant pieces from floating; therefore, in this method, the cells can be isolated very easily with high proliferation rate in comparison with the conventional methods.³³

MSCs Isolation with a Mixed Enzymatic-Explant Method

In the mixed procedure, after blood removal, vessels and the outer sheath of UC, the matrix is allowed to come into direct contact with the enzymatic solution to extract WJ. Then, WJ is cut into smaller pieces. In the next step, WJ pieces are placed in a culture medium with an enzymatic cocktail such as Collagenase or Hyaluronidase, followed by trypsin-Ethylene diamine tetra acetic acid (EDTA) at 37 °C in a humidified environment containing 5% CO₂ to loosen up the WJ without complete digestion. After the incubation period, the pieces are washed with PBS solution and transferred to a culture dish, where a similar procedure is repeated. In this method, the entire dish containing WJ pieces in either of the above-mentioned procedure is left undisturbed until MSCs migrate from WJ. By using mixed enzymatic-explant digestion, MSCs are isolated after 24-48 hours.¹⁶

Beravolu and others suggested that MSCs

isolated from cord-placenta junction (CPJ) have high proliferation potential and self-renew in vitro,²⁹ They also showed that the partially-digested perinatal tissue explants protocols are better because of less damage and higher amounts of homogeneous MSCs populations.²⁹

Robert Smith used a new closed system that decreased contaminant in mixed mechanical and enzymatic digestion. This protocol eliminates xenogenic supplement as well as the MSCs viability up to 90% after cryopreservation.²⁸

Comparison between Different MSCs Isolation Methods

Researchers have compared different MSCs isolation and proliferation methods. Studies have shown that cell isolation from the UC matrix using the 10-mm size tissue explant method causes shorter primary culture time, higher numbers of cells, and higher proliferation rates in comparison with the enzyme methods. In the explant method, much valuable time is wasted, but the price and biological contaminations are reduced and the number of isolated cells are high.³³

However, other characteristics such as immune phenotype and multilineage differentiation capacity did not differ significantly.³⁴ In the explanted method, the MSCs are isolated by migration. Due to MSC migratory and plastic adhesive properties, the isolation took approximately 5 days. Other studies had developed an optimized enzymatic isolation protocol within 3 hrs. Results showed that the population doubling time of the UC–MSC–Enzyme was estimated to be 2.76±0.60 days.³⁵

The explant method has several advantages in comparison with the enzymatic isolation. These advantages include putting no proteolytic

stress on cells, high yield isolation, and reduced cost and risk of biological contaminations.³³ Therefore, it has been shown that the explant method is very useful and effective amongst other methods (Table 1).

Hormones and Cytokines Induce MSC Differentiation into Mature Hepatocytes in Vitro

In order to induce MSC differentiation into mature hepatocytes in vitro, there are methods that provide adequate stimuli to maintain cellular function such as growth hormones, cytokines, extracellular matrix or co-culture with other cell types.³⁶ Several cytokines have an effect on cell growth and cell differentiation in to hepatocytes in vitro including hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), Oncostatin M (OSM), epidermal growth factor (EGF), transforming growth factor (TGF), insulin, insulin-like growth factor (IGF), leukemia inhibitory factor (LIF), and etc. Furthermore, the roles of chemical compounds such as norepinephrine, dexamethasone (Dex), nicotinamide (NTA), retinoic acid, sodium butyrate, and dimethylsulfoxide in promoting hepatic differentiation have been explored. Growth factors such as FGF and HGF secreted from mesoderm in the embryonic period are added to differentiated hepatocyte phenotype. Hence, several protocols have been proposed to facilitate MSC differentiation into hepatocytes with different degrees of differentiation.³⁶ During embryonic development, endodermal specification leads to the production of HGF and FGFs.³⁷

HGF is produced by stromal cells that promote motility, proliferation, invasion, morphogenesis, and survival hepatocytes. It is a potent mitogen and pleiotropic cytokine with

Table 1: Comparison between different MSCs isolation methods

MSCs isolation methods	Advantage	Disadvantage
Enzymatic procedure	-Can use the total length of the cord and MSC isolation from the three recognized compartments or can use some parts of UC ^{8, 13, 30} -Leads to an increase in stem cell yield without time-consuming producers ^{8, 30} -Isolation protocol within 3 hrs. ³⁵	-Proteolytic stress on cells -Increased price and -High risk of biological contaminations ³³
Explants procedure	-High proliferation rate -Higher numbers of homogeneous cell -The price and biological contaminations are reduced ³³ -no proteolytic stress on cells	-Explant pieces floating ³³ -The time need to isolate cells is loner ³⁵
Mixed enzymatic-explant digestion	-Shorter time need to isolate MSCs from tissue -High amounts of homogeneous cell. ²⁹ -MSCs viability up to 90 % after cryopreservation. ²⁸	-There are proteolytic stress on cells but is less than an enzymatic method -Price for enzymatic digestion also exists -High risk of biological contaminations ³³

a mesenchymal origin and plays a pivotal role in organ formation during embryogenesis and tissue homeostasis as well as liver regeneration in adults.^{38, 39} Combining HGF and FGF initiates the related gene expression to different hepatocyte from MSCs. FGF cluster has a direct effect on cell growth, embryonic development, tissue restoration, and morphogenesis.³⁶ A study showed that by pre-exposing cells to FGF4 before treating with IGF-1 and HGF, liver-specific markers increased.⁴⁰ Albumin was expressed more in the control cultures, but cytokeratin 18 was expressed more in FGF4-treated cells.⁴¹

Another cytokine that promotes liver cell growth is OSM. It is a member of the interleukin 6 family cytokines. In spite of its failure to induce hepatocyte-like phenotype, OSM acts as a significant paracrine regulator in the hepatocyte development toward maturation.⁴²

Dex, a synthetic glucocorticoid, is a potent glucocorticoid receptor (GR) activator, which is a member of the nuclear hormone receptor superfamily. GR regulates genes controlling, metabolism, and immune response. It is binding to specific response elements and that leads to the expression of anti-inflammatory and immunosuppressant reactions manifested clearly through the repression of the NFκB and AP-1 pathways.⁴³ Dex stimulates the hepatocyte to form a cuboidal phenotypic architecture in vitro while accommodating the expression of liver-enriched transcription factors such as C/EBPα, HFN-4α, and RXRα.⁴⁴ Furthermore, Dex suppresses the hepatocyte proliferative state otherwise stimulated by growth factors such as EGF.⁴⁵ Dex exerts depends on the results of hepatocyte culture and differentiation, hence high doses of it leads to proliferation,⁴⁶ but low concentrations of it induces hepatic lineage differentiation.⁴⁷⁻⁴⁹

Hepatic Differentiation Methods

In regenerative medicine and liver tissue engineering, one of the most important issues is hepatic differentiation. As a result, different protocols have been examined that can be categorized into two groups of cocktail and sequential.⁵⁰ Several sequential methods have been examined for hepatic differentiation. Some studies have reported hepatic differentiation protocols in four, three, and two steps.⁵¹⁻⁵³ For example, the following method was explained by Yoon and others (2010). Mesenchymal stem cells were differentiated by a four-step protocol:

step1: DMEM low glucose (LG)+10 ng/ml FGF-4 for 2 days

step2: DMEM (LG)+1%ITS+20 ng/ml HGF for

2 days

step3: DMEM (LG)+100nMDexamethasone +1%ITS+100 nM Glucagon+10ng/ml OSM for 2 days

step4: DMEM (LG)+100 nM Dexamethasone +1%ITS+100 nM Glucagon+10 ng/ml+ 1%DMSO or100 nM TSA for 8 days. The medium was exchanged every two days.⁵²

The secreted molecules from human MSCs as well as growth factors in the medium affected hepatocyte-like cells differentiation.⁵⁴

A recent study showed that stem cells with certain microRNAs (miRNAs) could be differentiated into functional hepatocyte-like cells; however, such investigations should be approved in the future studies.⁵⁵ Takagi and others have introduced and optimized five-miRNA combinations to differentiate MSC-derived hepatocyte-like cells during 7 days.⁵⁶ Table 2 and figure 1 include various parts, such as stem cells isolation protocols, various differentiation protocols (2-4 steps or cocktail), different culturing methods (2D, 3D), and results.

Hepatocyte Differentiation in 3-Dimensional Culture

In recent years, several studies have been conducted on 3-dimensional (3-D) differentiation of mesenchymal stem cell, using biocompatible scaffolds such as alginate, collagen, gelatin, and peptide-Gly-Leu-amide (PGLA). Scaffold encapsulation system in vitro culture has been described in both the induction of adult stem cell differentiation and increased function of mature hepatocytes, that can enhance intercellular contacts, improve cell migration, and mimic an in vivo environment condition. 3-D cell culture condition improves the cell stability as well as influencing gene expression pattern.

Several studies have indicated that 3-D co-culture of hepatocytes with different cell types mimics the in vivo, which improves hepatocyte viability and function in vitro.⁶⁹ The 3D scaffold system did not change the expression level of the cytoskeletal elements CK-18 and 19, but the expression of claudine as a tight junction marker increased significantly in 3D scaffold system.⁷⁰

Another study showed that the gelatin cryogel scaffold could improve hADMSCs differentiation capability into hepatocyte-like cells in comparison with 2D tissue culture polystyrene. In this scaffold cell number, morphology and viability increased. The gene expression of hepatocytes pacific genes (*ALB*, *AFP*, *CK-18*, and *CK-19*), urea production, and glycogen storage were reported to have improved.⁷¹

In addition, liver expression markers and

Table 2: In vitro protocols of MSCs isolation and differentiation in- to-hepatocyte-like cells

Isolation of MSCs	Induction growth factors	Methods of hepatic differentiation	Culture (2D/3D)	References
Enzymatic	Animal model In vivo differentiation	Animal model In vivo differentiation	3D In-vivo	Chen et al. 2016 ¹²
Result: Transplanted hUCMSCs into hepatectomized rats differentiated into hepatocyte-like cells				
Enzymatic	EGF and bFGF, HGF, bFGF and nicotinamide, Dex, ITS premix and OSM	3 steps protocol	2D	Lee, et al. 2012 ¹³
Result: Potential for hepatogenic differentiation of placenta-derived stem cells (PDSCs) containing WJ				
Explant	HGF,FGF-4,OSM, Dex 2	2 steps protocol	2D	Esmaeli 2014 ¹⁵
Result: Shift in the fatty acid profile during the hepatocyte differentiation				
Enzymatic	EGF, bFGF (2 days) HGF, bFGF (10 days) nicotinamide, ITS (10 days)	Sequential 3 steps	2D	Campard, et al. 2008 ³¹
Result: Human MSCs can be considered as a source with hepatogenic potential				
Explant	bFGF, EGF,	Cocktail	3D	Su et al. 2014 ³²
Result: Poly (3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) scaffolds loaded with UC-MSCs or differentiated UC-MSCs promoted the recovery of injured livers				
Enzymatic	DMEM/F-12 medium with HGF, EGF, ITS, Dex, OSM	2 steps protocol	2D	Zhou et al. 2014 ⁵⁷
Result: Stimulation of host hepatocyte regeneration via hUCMSCs				
Explant	40 ng/mL HGF And 10 ng/mL FGF-4	Cocktail	2D	Zhang et al. 2009 ⁵⁸
Result: UC-MSC can differentiate into functional hepatocyte-like cells and have advantages over BM-MSC				
Explant	EGF and FGF4, HGF, FGF4, Dex, ITS and OSM	2 steps protocol	3D	Chitrangi et al. 2017 ⁵⁹
Result: gelatin-vinyl acetate scaffold enhanced differentiation of UC-MSCs to hepatocyte-like cells				
Ex-vivo	-	Ex vivo	-	Gomez-Aristizabl and Davies 2012 ⁶⁰
Result: Human UC perivascular Cells (hucpvcs) can act as stromal cells for rat hepatocytes,				
Explant	10 ng/mL HGF, 10 ng/mL FGF4	Cocktail	2D	Ewida, et al. 2016 ⁶¹
Result: hMSCs can differentiate <i>in vitro</i> into functional hLCs (hepatocyte-like stem cells) in a liver fibrosis rat model				
Explant	Liver homogenate supernatants (LHS)	Cocktail	2D	Xue, et al. 2016 ⁶²
Result: Liver tissue microenvironment may contribute to the differentiation of hUCMSCs into hepatocytes both in vitro and in vivo				
Enzymatic	Dex, EGF, HGF, ITS and OSM	2-step (for 2 weeks)	2D	Liang, et al. 2012 ⁶³
Result: Extending hUCMSCs replicative lifespan without influencing hepatogenic differentiation potential via telomerization of hUCMSCs by human telomerase reverse transcriptase (HTERT)				
Explant	FGF-4 and HGF(14 days) HGF, ITS, oncostatin and Dex. (14 days)	Sequential 2 steps	3D	Raut, and Khanna 2016 ⁶⁴
Result: improved expression of hepatocyte-specific miRNAs, miR-23b cluster (miR-27b-3p, miR-24-1-5p and miR-23b-3p), miR-30a-5p, miR-26a-5p, miR-148a-3p, miR-192-5p, miR-122-5p due to VPA pre-treatment				
Explant	ITS, HGF, OSM, Dex (15 days).	Cocktail	2D	An, et al. 2014 ⁶⁵
Result: The lineage conversion of hUCMSCs to hepatic cell fate by upregulating the expression of endodermal genes through AKT and ERK activation by valproic acid (VPA)				
Explant	EGF, bFGF, HGF, bFGF nicotinamide, OSM, ITS	2 steps protocol	2D	Zheng, et al. 2015 ⁶⁶
Result: Oncostatin M (OSM) acts an important role in hepatogenic differentiation.				
Explant	HGF, infection with a lentivirus containing a miRNA inhibitor sequence	Cocktail	2D	Cui, et al. 2013 ⁶⁷
Result: The capability of miRNAs to converting hMSCs to a hepatocyte phenotype in vitro				
Explant	IGF-I, HGF and Dex OSM up to 23 days	2 steps	3D cell aggregate	Talaei-Khozani, et al. 2015 ⁶⁸
Result: Facilitating hepatocyte differentiation may be by the UCMSCs aggregate formation before administration of the differentiation protocols				

HGF: hepatocyte growth factor; bFGF: basic fibroblast growth factor; OSM: Oncostatin M; Dex: dexamethasone; EGF: epidermal growth factor; ITS: insulin–transferrin–selenium; IGF: insulin-like growth factor; TGF: transforming growth factor; DMEM: Dulbecco's Modified Eagle's Medium

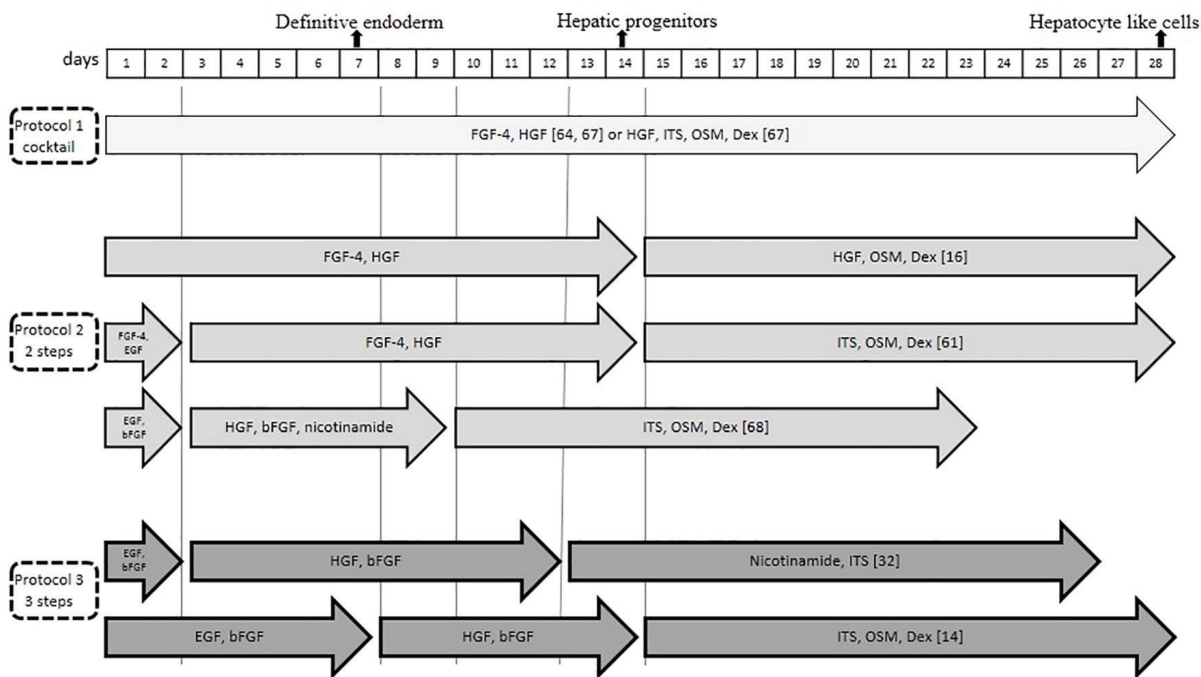


Figure 1: The figure depicts the in vitro protocols of MSCs differentiation into hepatocyte-like cells

HNF4α showed an improvement in 3-D culture in comparison with 2-D.⁷²

Discussion

Stem cells represent a unique source of self-renewing organism. Stem cell therapy has the potential to become an alternative therapy for liver transplantation. Several important factors, such as stem cell source, cytokine, hormone, differentiation protocols, and culture system, should be considered for hepatic differentiation of stem cells into hepatocyte-like cells. Hepatic differentiation is often linked to the selection of stem cell source. Hence, UC-MSCs have been introduced as a suitable stem cells source. Several studies have shown that UC-MSCs with similar immune phenotype and multilineage differentiation have higher potential to expand in comparison with other sources such as BM-MSCs and ADMSCs.^{9, 10, 25, 26}

Selecting the best isolation method is critical since it saves time, reduces costs, and increases the cell expansion and proliferation. There are still ongoing studies on these methods. Most of these studies have reported that the explant method could be considered as a suitable alternative. A recent review showed that using explant method could lead to shorter primary culture time, higher numbers of cells, and higher proliferation rates in comparison with enzyme methods. Additionally, the price and biological

contaminations were reduced as well.³³

In this review, various differentiation protocols of stem cells into hepatocyte-like cells are summarized in table 2.

One of the most important factors in hepatic differentiation is to determine an appropriate induction method. Several hormones and cytokines including HGF, FGF, OSM, EGF, TGF, IGF, LIF, and ... have known to be involved in the regeneration of liver after hepatic injury and during organogenesis. Combining these cytokines initiates the related genes expression, to differentiate MSCs into hepatocyte form. During organogenesis, liver development is a sequential array of distinct biological events. Therefore, several studies have been conducted on the use of cell cytokines, such as HGF, FGF, and oncostatin M, in a sequential manner in 3 or 4 stages.

The culture system method (2D and 3D) is very important. It has been shown that 2D culture of stem cell in the first stage of differentiation is critical, and, in the second stage, 3D culture system can enhance stem cell differentiation into hepatocyte. The 3D culture system can mimic in vivo condition. Moreover, the differentiated hepatocyte has the best quality and function. Several studies investigated hepatic differentiation with cytokines in 2D and 3D culture, and the results revealed more advantages for 3D culture conditions than 2D culture conditions.^{69, 70}

Conclusion

In this review, we conclude that WJ-MSC can be considered as a suitable source for differentiation into hepatocyte-like cells since it has no ethical concern but high proliferation and low immunogenicity. WJ-MSC isolation from the umbilical cord is very efficient through explant culture method in comparison with enzymatic methods. In the early stage of differentiation, 2D culture is more useful, but in the development stage, 3D culture system with HGF and FGF cytokines can be more effective in the differentiation process. In this review, we attempted to highlight all factors in hepatocyte differentiation, but the best and most effective protocol for hepatocyte differentiation is still unknown.

Acknowledgement

The authors wish to thank the staff at Cellular and Molecular Research Center of Ahvaz Jundishapur University of Medical Sciences for their support. The authors also wish to thank Mr. H. Argasi at the Research Consultation Center (RCC) of Shiraz University of Medical Sciences for his invaluable assistance in editing this manuscript.

Conflict of Interest: None declared.

References

- Sun K, Xie X, Xie J, Jiao S, Chen X, Zhao X, et al. Cell-based therapy for acute and chronic liver failures: distinct diseases, different choices. *Sci Rep.* 2014;4:6494. doi: 10.1038/srep06494. PubMed PMID: 25263068; PubMed Central PMCID: PMC4178291.
- Russo FP, Parola M. Stem cells in liver failure. *Best Pract Res Clin Gastroenterol.* 2012;26:35-45. doi: 10.1016/j.bpg.2012.01.001. PubMed PMID: 22482524.
- Schwartz RE, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, et al. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. *J Clin Invest.* 2002;109:1291-302. doi: 10.1172/JCI15182. PubMed PMID: 12021244; PubMed Central PMCID: PMC150983.
- Seo MJ, Suh SY, Bae YC, Jung JS. Differentiation of human adipose stromal cells into hepatic lineage in vitro and in vivo. *Biochem Biophys Res Commun.* 2005;328:258-64. doi: 10.1016/j.bbrc.2004.12.158. PubMed PMID: 15670778.
- Sidhu KS. *Frontiers in Pluripotent Stem Cells Research and Therapeutic Potentials: Bench-to-bedside.* Sharjah: Bentham Science Publishers; 2012.
- Kolios G, Moodley Y. Introduction to stem cells and regenerative medicine. *Respiration.* 2013;85:3-10. doi: 10.1159/000345615. PubMed PMID: 23257690.
- Romanov YA, Svintsitskaya VA, Smirnov VN. Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. *Stem Cells.* 2003;21:105-10. doi: 10.1634/stemcells.21-1-105. PubMed PMID: 12529557.
- Seshareddy K, Troyer D, Weiss ML. Method to isolate mesenchymal-like cells from Wharton's Jelly of umbilical cord. *Methods Cell Biol.* 2008;86:101-19. doi: 10.1016/S0091-679X(08)00006-X. PubMed PMID: 18442646.
- Simoies IN, Boura JS, dos Santos F, Andrade PZ, Cardoso CM, Gimble JM, et al. Human mesenchymal stem cells from the umbilical cord matrix: successful isolation and ex vivo expansion using serum-/xeno-free culture media. *Biotechnol J.* 2013;8:448-58. doi: 10.1002/biot.201200340. PubMed PMID: 23420807.
- Cheng H, Qiu L, Ma J, Zhang H, Cheng M, Li W, et al. Replicative senescence of human bone marrow and umbilical cord derived mesenchymal stem cells and their differentiation to adipocytes and osteoblasts. *Mol Biol Rep.* 2011;38:5161-8. doi: 10.1007/s11033-010-0665-2. PubMed PMID: 21188535.
- Deuse T, Stubbendorff M, Tang-Quan K, Phillips N, Kay MA, Eiermann T, et al. Immunogenicity and immunomodulatory properties of umbilical cord lining mesenchymal stem cells. *Cell Transplant.* 2011;20:655-67. doi: 10.3727/096368910X536473. PubMed PMID: 21054940.
- Chen Z, Kuang Q, Lao XJ, Yang J, Huang W, Zhou D. Differentiation of UC-MSCs into hepatocyte-like cells in partially hepatectomized model rats. *Exp Ther Med.* 2016;12:1775-9. doi: 10.3892/etm.2016.3543. PubMed PMID: 27602090; PubMed Central PMCID: PMC4998204.
- Lee HJ, Jung J, Cho KJ, Lee CK, Hwang SG, Kim GJ. Comparison of in vitro hepatogenic differentiation potential between various placenta-derived stem cells and other adult stem cells as an alternative source of functional hepatocytes. *Differentiation.* 2012;84:223-31. doi: 10.1016/j.diff.2012.05.007. PubMed PMID: 22885322.
- Hashemitabar M, Allahbakhshi E, Tabande MR, Orazizadeh M, Dehbashi FN, Azandeh

- S, et al. Isolation and Characterization of Human Umbilical Cord Mesenchymal Stem Cells and Their Differentiation into Pdx-1+ Cells. *J Biomed Sci Eng.* 2015;8:780-8. doi: 10.4236/jbise.2015.811074.
- 15 Esmaeli S, Allameh A, Soleimani M, Rahbarizadeh F, Frouzandeh-Moghadam M. The role of albumin and PPAR-alpha in differentiation-dependent change of fatty acid profile during differentiation of mesenchymal stem cells to hepatocyte-like cells. *Cell Biochem Funct.* 2014;32:410-9. doi: 10.1002/cbf.3031. PubMed PMID: 24585388.
 - 16 Azandeh S, Orazizadeh M, Hashemitabar M, Khodadadi A, Shayesteh AA, Nejad DB, et al. Mixed enzymatic-explant protocol for isolation of mesenchymal stem cells from Wharton's jelly and encapsulation in 3D culture system. *Journal of Biomedical Science and Engineering.* 2012;5:580. doi: 10.4236/jbise.2012.510071.
 - 17 Sarugaser R, Lickorish D, Baksh D, Hosseini MM, Davies JE. Human umbilical cord perivascular (HUCPV) cells: a source of mesenchymal progenitors. *Stem Cells.* 2005;23:220-9. doi: 10.1634/stemcells.2004-0166. PubMed PMID: 15671145.
 - 18 Ruetze M, Gallinat S, Lim IJ, Chow E, Phan TT, Staeb F, et al. Common features of umbilical cord epithelial cells and epidermal keratinocytes. *J Dermatol Sci.* 2008;50:227-31. doi: 10.1016/j.jdermsci.2007.12.006. PubMed PMID: 18242061.
 - 19 Ilancheran S, Michalska A, Peh G, Wallace EM, Pera M, Manuelpillai U. Stem cells derived from human fetal membranes display multilineage differentiation potential. *Biol Reprod.* 2007;77:577-88. doi: 10.1095/biolreprod.106.055244. PubMed PMID: 17494917.
 - 20 Weiss ML, Medicetty S, Bledsoe AR, Rachakatla RS, Choi M, Merchav S, et al. Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. *Stem Cells.* 2006;24:781-92. doi: 10.1634/stemcells.2005-0330. PubMed PMID: 16223852.
 - 21 Fong CY, Richards M, Manasi N, Biswas A, Bongso A. Comparative growth behaviour and characterization of stem cells from human Wharton's jelly. *Reprod Biomed Online.* 2007;15:708-18. PubMed PMID: 18062871.
 - 22 Fong CY, Chak LL, Biswas A, Tan JH, Gauthaman K, Chan WK, et al. Human Wharton's jelly stem cells have unique transcriptome profiles compared to human embryonic stem cells and other mesenchymal stem cells. *Stem Cell Rev.* 2011;7:1-16. doi: 10.1007/s12015-010-9166-x. PubMed PMID: 20602182.
 - 23 Lee BH, Li B, Guelcher SA. Gel microstructure regulates proliferation and differentiation of MC3T3-E1 cells encapsulated in alginate beads. *Acta Biomater.* 2012;8:1693-702. doi: 10.1016/j.actbio.2012.01.012. PubMed PMID: 22306825; PubMed Central PMCID: PMC3314144.
 - 24 Fong CY, Subramanian A, Biswas A, Gauthaman K, Srikanth P, Hande MP, 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. doi: 10.1016/j.rbmo.2010.04.010. PubMed PMID: 20638335.
 - 25 Rao MS, Mattson MP. Stem cells and aging: expanding the possibilities. *Mech Ageing Dev.* 2001;122:713-34. PubMed PMID: 11322994. doi: 10.1016/S0047-6374(01)00224-X.
 - 26 Huss R. Isolation of primary and immortalized CD34-hematopoietic and mesenchymal stem cells from various sources. *Stem Cells.* 2000;18:1-9. doi: 10.1634/stemcells.18-1-1. PubMed PMID: 10661567.
 - 27 Manca MF, Zwart I, Beo J, Palasingham R, Jen LS, Navarrete R, et al. Characterization of mesenchymal stromal cells derived from full-term umbilical cord blood. *Cytotherapy.* 2008;10:54-68. doi: 10.1080/14653240701732763. PubMed PMID: 18202975.
 - 28 Smith JR, Cromer A, Weiss ML. Human Umbilical Cord Mesenchymal Stromal Cell Isolation, Expansion, Cryopreservation, and Characterization. *Curr Protoc Stem Cell Biol.* 2017;41:1F 18 1-1F 23. doi: 10.1002/cpsc.24. PubMed PMID: 28510332.
 - 29 Beeravolu N, McKee C, Alamri A, Mikhael S, Brown C, Perez-Cruet M, et al. Isolation and Characterization of Mesenchymal Stromal Cells from Human Umbilical Cord and Fetal Placenta. *J Vis Exp.* 2017. doi: 10.3791/55224. PubMed PMID: 28447991; PubMed Central PMCID: PMC5564456.
 - 30 Tsgias N, Koliakos I, Karagiannis V, Eleftheriadou M, Koliakos GG. Isolation of mesenchymal stem cells using the total length of umbilical cord for transplantation purposes. *Transfus Med.* 2011;21:253-61. doi: 10.1111/j.1365-3148.2011.01076.x. PubMed PMID: 21623971.
 - 31 Campard D, Lysy PA, Najimi M, Sokal EM. Native umbilical cord matrix stem cells express hepatic markers and differentiate

- into hepatocyte-like cells. *Gastroenterology*. 2008;134:833-48. doi: 10.1053/j.gastro.2007.12.024. PubMed PMID: 18243183.
- 32 Su Z, Li P, Wu B, Ma H, Wang Y, Liu G, et al. PHBVHHx scaffolds loaded with umbilical cord-derived mesenchymal stem cells or hepatocyte-like cells differentiated from these cells for liver tissue engineering. *Mater Sci Eng C Mater Biol Appl*. 2014;45:374-82. doi: 10.1016/j.msec.2014.09.022. PubMed PMID: 25491842.
 - 33 Hendijani F. Explant culture: An advantageous method for isolation of mesenchymal stem cells from human tissues. *Cell Prolif*. 2017;50. doi: 10.1111/cpr.12334. PubMed PMID: 28144997.
 - 34 Hua J, Gong J, Meng H, Xu B, Yao L, Qian M, et al. Comparison of different methods for the isolation of mesenchymal stem cells from umbilical cord matrix: proliferation and multilineage differentiation as compared to mesenchymal stem cells from umbilical cord blood and bone marrow. *Cell Biol Int*. 2013. doi: 10.1002/cbin.10188. PubMed PMID: 24123669.
 - 35 Buyl K, Vanhaecke T, Desmae T, Lagneaux L, Rogiers V, Najar M, et al. Evaluation of a new standardized enzymatic isolation protocol for human umbilical cord-derived stem cells. *Toxicol In Vitro*. 2015;29:1254-62. doi: 10.1016/j.tiv.2014.12.008. PubMed PMID: 25541070.
 - 36 Wu X-B, Tao R. Hepatocyte differentiation of mesenchymal stem cells. *Hepatobiliary Pancreat Dis Int*. 2012;11:360-71. doi: 10.1016/S1499-3872(12)60193-3.
 - 37 Jung J, Zheng M, Goldfarb M, Zaret KS. Initiation of mammalian liver development from endoderm by fibroblast growth factors. *Science*. 1999;284:1998-2003. PubMed PMID: 10373120.
 - 38 Santos OF, Barros EJ, Yang XM, Matsumoto K, Nakamura T, Park M, et al. Involvement of hepatocyte growth factor in kidney development. *Dev Biol*. 1994;163:525-9. doi: 10.1006/dbio.1994.1169. PubMed PMID: 8200486.
 - 39 Uehara Y, Minowa O, Mori C, Shiota K, Kuno J, Noda T, et al. Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor. *Nature*. 1995;373:702-5. doi: 10.1038/373702a0. PubMed PMID: 7854453.
 - 40 Talaei-Khozani T. Improved Differentiation of Mesenchymal Stem Cells into Hepatocyte-like Cells using FGF4 and IGF-1 in 3D culture. *Int J Stem Cell Res Transplant*. 2015;3:109-17. doi: 10.19070/2328-3548-1500018.
 - 41 Vojdani Z, Khodabandeh Z, Jaberipour M, Hosseini A, Bahmanpour S, Talaei-Khozani T. The influence of fibroblast growth factor 4 on hepatogenic capacity of Wharton's jelly mesenchymal stromal cells. *Rom J Morphol Embryol*. 2015;56:1043-50. PubMed PMID: 26662137.
 - 42 Kinoshita T, Sekiguchi T, Xu MJ, Ito Y, Kamiya A, Tsuji K, et al. Hepatic differentiation induced by oncostatin M attenuates fetal liver hematopoiesis. *Proc Natl Acad Sci U S A*. 1999;96:7265-70. PubMed PMID: 10377403; PubMed Central PMCID: PMCPMC22074.
 - 43 Nelson G, Wilde GJ, Spiller DG, Kennedy SM, Ray DW, Sullivan E, et al. NF-kappaB signalling is inhibited by glucocorticoid receptor and STAT6 via distinct mechanisms. *J Cell Sci*. 2003;116:2495-503. doi: 10.1242/jcs.00461. PubMed PMID: 12734399.
 - 44 Dasgupta A, Hughey R, Lancin P, Larue L, Moghe PV. E-cadherin synergistically induces hepatospecific phenotype and maturation of embryonic stem cells in conjunction with hepatotrophic factors. *Biotechnol Bioeng*. 2005;92:257-66. doi: 10.1002/bit.20676. PubMed PMID: 16167333.
 - 45 Scheving LA, Stevenson MC, Zhang X, Russell WE. Cultured rat hepatocytes upregulate Akt and ERK in an ErbB-2-dependent manner. *Am J Physiol Gastrointest Liver Physiol*. 2008;295:G322-31. doi: 10.1152/ajpgi.00597.2007. PubMed PMID: 18535289; PubMed Central PMCID: PMCPMC2519852.
 - 46 Scheving LA, Buchanan R, Krause MA, Zhang X, Stevenson MC, Russell WE. Dexamethasone modulates ErbB tyrosine kinase expression and signaling through multiple and redundant mechanisms in cultured rat hepatocytes. *Am J Physiol Gastrointest Liver Physiol*. 2007;293:G552-9. doi: 10.1152/ajpgi.00140.2007. PubMed PMID: 17585012.
 - 47 Baharvand H, Hashemi SM, Shahsavani M. Differentiation of human embryonic stem cells into functional hepatocyte-like cells in a serum-free adherent culture condition. *Differentiation*. 2008;76:465-77. doi: 10.1111/j.1432-0436.2007.00252.x. PubMed PMID: 18177418.
 - 48 Saito K, Yoshikawa M, Ojui Y, Moriya K, Nishiofuku M, Ueda S, et al. Promoted differentiation of cynomolgus monkey ES cells into hepatocyte-like cells by co-culture with mouse fetal liver-derived cells. *World J Gastroenterol*. 2006;12:6818-27. PubMed PMID: 17106931; PubMed Central PMCID:

- PMCPMC4087437.
- 49 Shirahashi H, Wu J, Yamamoto N, Catana A, Wege H, Wager B, et al. Differentiation of human and mouse embryonic stem cells along a hepatocyte lineage. *Cell Transplant*. 2004;13:197-211. PubMed PMID: 15191158.
 - 50 Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, Quinn G, et al. Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes. *Hepatology*. 2007;46:219-28. doi: 10.1002/hep.21704. PubMed PMID: 17596885.
 - 51 Snykers S, De Kock J, Tamara V, Rogiers V. Hepatic differentiation of mesenchymal stem cells: in vitro strategies. *Methods Mol Biol*. 2011;698:305-14. doi: 10.1007/978-1-60761-999-4_23. PubMed PMID: 21431528.
 - 52 Yoon H-H, Jung B-Y, Seo Y-K, Song K-Y, Park J-K. In vitro hepatic differentiation of umbilical cord-derived mesenchymal stem cell. *Process Biochemistry*. 2010;45:1857-64. doi: 10.1016/j.procbio.2010.06.009.
 - 53 Lee KD, Kuo TKC, Whang Peng J, Chung YF, Lin CT, Chou SH, et al. In vitro hepatic differentiation of human mesenchymal stem cells. *Hepatology*. 2004;40:1275-84. doi: 10.1002/hep.20469.
 - 54 Takagi C, Yagi H, Hieda M, Tajima K, Hibi T, Abe Y, et al. Mesenchymal Stem Cells Contribute to Hepatic Maturation of Human Induced Pluripotent Stem Cells. *Eur Surg Res*. 2017;58:27-39. doi: 10.1159/000448516. PubMed PMID: 27598993.
 - 55 Davoodian N, Lotfi AS, Soleimani M, Ghaneialvar H. The combination of miR-122 overexpression and Let-7f silencing induces hepatic differentiation of adipose tissue-derived stem cells. *Cell Biol Int*. 2017;41:1083-92. doi: 10.1002/cbin.10836. PubMed PMID: 28792091.
 - 56 Zhou X, Cui L, Zhou X, Yang Q, Wang L, Guo G, et al. Induction of hepatocyte-like cells from human umbilical cord-derived mesenchymal stem cells by defined microRNAs. *J Cell Mol Med*. 2017;21:881-93. doi: 10.1111/jcmm.13027. PubMed PMID: 27874233; PubMed Central PMCID: PMCPMC5387126.
 - 57 Zhou R, Li Z, He C, Li R, Xia H, Li C, et al. Human umbilical cord mesenchymal stem cells and derived hepatocyte-like cells exhibit similar therapeutic effects on an acute liver failure mouse model. *PLoS One*. 2014;9:e104392. doi: 10.1371/journal.pone.0104392. PubMed PMID: 25101638; PubMed Central PMCID: PMCPMC4125182.
 - 58 Zhang YN, Lie PC, Wei X. Differentiation of mesenchymal stromal cells derived from umbilical cord Wharton's jelly into hepatocyte-like cells. *Cytotherapy*. 2009;11:548-58. doi: 10.1080/14653240903051533. PubMed PMID: 19657806.
 - 59 Chitrangi S, Nair P, Khanna A. Three-dimensional polymer scaffolds for enhanced differentiation of human mesenchymal stem cells to hepatocyte-like cells: a comparative study. *J Tissue Eng Regen Med*. 2017;11:2359-72. doi: 10.1002/term.2136. PubMed PMID: 27121646.
 - 60 Gomez-Aristizabal A, Davies JE. Human umbilical cord perivascular cells improve rat hepatocyte function ex vivo. *Tissue Eng Part A*. 2012;18:2487-96. doi: 10.1089/ten.TEA.2011.0669. PubMed PMID: 22731670.
 - 61 Ewida SF, Abdou AG, El-Rasol Elhosary AA, El-Ghane Metawe SA. Hepatocyte-like Versus Mesenchymal Stem Cells in CCl4-induced Liver Fibrosis. *Appl Immunohistochem Mol Morphol*. 2017;25:736-45. doi: 10.1097/PAI.0000000000000373. PubMed PMID: 27153445.
 - 62 Xue G, Han X, Ma X, Wu H, Qin Y, Liu J, et al. Effect of Microenvironment on Differentiation of Human Umbilical Cord Mesenchymal Stem Cells into Hepatocytes In Vitro and In Vivo. *Biomed Res Int*. 2016;2016:8916534. doi: 10.1155/2016/8916534. PubMed PMID: 27088093; PubMed Central PMCID: PMCPMC4764721.
 - 63 Liang XJ, Chen XJ, Yang DH, Huang SM, Sun GD, Chen YP. Differentiation of human umbilical cord mesenchymal stem cells into hepatocyte-like cells by hTERT gene transfection in vitro. *Cell Biol Int*. 2012;36:215-21. doi: 10.1042/CBI20110350. PubMed PMID: 21988655.
 - 64 Raut A, Khanna A. Enhanced expression of hepatocyte-specific microRNAs in valproic acid mediated hepatic trans-differentiation of human umbilical cord derived mesenchymal stem cells. *Exp Cell Res*. 2016;343:237-47. doi: 10.1016/j.yexcr.2016.03.015. PubMed PMID: 27001466.
 - 65 An SY, Han J, Lim HJ, Park SY, Kim JH, Do BR, et al. Valproic acid promotes differentiation of hepatocyte-like cells from whole human umbilical cord-derived mesenchymal stem cells. *Tissue Cell*. 2014;46:127-35. doi: 10.1016/j.tice.2013.12.006. PubMed PMID: 24472423.
 - 66 Zheng G, Liu Y, Jing Q, Zhang L. Differentiation of human umbilical cord-derived mesenchymal stem cells into hepatocytes in vitro. *Biomed Mater Eng*. 2015;25:145-57. doi: 10.3233/BME-141249. PubMed PMID: 25538065.
 - 67 Cui L, Shi Y, Zhou X, Wang X, Wang J, Lan Y, et al. A set of microRNAs mediate

- direct conversion of human umbilical cord lining-derived mesenchymal stem cells into hepatocytes. *Cell Death Dis.* 2013;4:e918. doi: 10.1038/cddis.2013.429. PubMed PMID: 24232094; PubMed Central PMCID: PMC3847311.
- 68 Talaei-Khozani T, Borhani-Haghighi M, Ayatollahi M, Vojdani Z. An in vitro model for hepatocyte-like cell differentiation from Wharton's jelly derived-mesenchymal stem cells by cell-base aggregates. *Gastroenterol Hepatol Bed Bench.* 2015;8:188-99. PubMed PMID: 26328041; PubMed Central PMCID: PMC3847311.
- 69 Azandeh S, Mohammad Gharravi A, Orzizadeh M, Khodadi A, Hashemi Tabar M. Improvement of mesenchymal stem cell differentiation into the endoderm lineage by four step sequential method in biocompatible biomaterial. *Bioimpacts.* 2016;6:9-13. doi: 10.15171/bi.2016.02. PubMed PMID: 27340619; PubMed Central PMCID: PMC4916552.
- 70 Khodabandeh Z, Vojdani Z, Talaei-Khozani T, Jaberipour M, Hosseini A, Bahmanpour S. Comparison of the Expression of Hepatic Genes by Human Wharton's Jelly Mesenchymal Stem Cells Cultured in 2D and 3D Collagen Culture Systems. *Iran J Med Sci.* 2016;41:28-36. PubMed PMID: 26722142; PubMed Central PMCID: PMC4691267.
- 71 Ghaderi Gandomani M, Sahebghadam Lotfi A, Kordi Tamandani D, Arjmand S, Alizadeh S. The enhancement of differentiating adipose derived mesenchymal stem cells toward hepatocyte like cells using gelatin cryogel scaffold. *Biochem Biophys Res Commun.* 2017;491:1000-6. doi: 10.1016/j.bbrc.2017.07.167. PubMed PMID: 28778389.
- 72 Talaei-Khozani T, Khodabandeh Z, Jaberipour M, Hosseini A, Bahmanpour S, Vojdani Z. Comparison of hepatic nuclear factor-4 expression in two- and three-dimensional culture of Wharton's jelly-derived cells exposed to hepatogenic medium. *Rom J Morphol Embryol.* 2015;56:1365-70. PubMed PMID: 26743282.