Influential factors for optimizing and strengthening mesenchymal stem cells and hematopoietic stem cells co-culture

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Abstract

Mesenchymal stem cells [MSCs] and Hematopoietic stem cells [HSCs] are two types of bone marrow stem cells that could proliferate and differentiate into different cell lineages. HSCs interact with MSCs under the protective conditions, called niche. Numerous studies have indicated supportive effects of MSCs on HSCs proliferation and differentiation. Furthermore, HSCs have many clinical applications and could treat different hematologic and non-hematologic diseases. For this purpose, there is a need to perform *in vitro* studies to optimize their expansion. Therefore, various methods including co-culture with MSCs are used to address the limitations of HSCs culture. Some parameters that might be effective for improving the co-culture system, such as MSC paracrine profile, scaffolds, hypoxia, culture medium additives, and the use of various MSC sources, have been examined in different studies. In this article, we investigated the potential factors for optimizing HSCs/ MSCs co-culture. It might be helpful to apply a suitable approach for providing high quality HSCs and improving their therapeutic applications in the required fields.

Background

Stem cells are primitive cells with at least two characteristics, including self-renewal and differentiation into various lineages [1]. There are two main populations of stem cells in the bone marrow, the hematopoietic stem cells (HSCs) and the mesenchymal stromal cells (MSCs) [2]. MSCs are valuable cells that could support other bone marrow cells by their close relationship with them [1]. These cells also play an essential role in the proliferation, survival, and differentiation of HSCs through the secretion of growth factors and cell-cell contact. Moreover, MSCs can be used as a feeder layer in HSCs culture [3].

MSCs are plastic-adhesive spindle-shaped cells that expanded in specific culture media *in vitro* and are one of the most critical supporting cells for the hematopoiesis system *in vivo* [4, 1]. These cells express CD73, CD90, CD105, CD16, and HLA-ABC and do not express CD31, CD34, CD45, CD80, and HLA-DR [1, 5, 6]. MSCs are progenitor cells that can differentiate into different cells under specific conditions [6, 7].

MSCs can be isolated from different tissues, including adipose tissue, amniotic fluid [6], bone marrow, liver, lung, spleen [8], placenta, umbilical cord blood [9], Wharton jelly, and other sources. MSC secretes cytokines, chemokines, and growth factors [10]. These properties can be utilized in the disease treatment and regenerative medicine [6, 10]. The MSC-secreted cytokines, including Interleukin 6 (IL-6), FMS-like tyrosine kinase 3 ligand (FLT3L), stem cell factor (SCF), granulocyte colony-stimulating factor (G-CSF), and granulocyte monocyte colony-stimulating factor (GM-CSF), involve in HSCs differentiation and proliferation [11].

MSCs are intrinsically capable of secreting other cytokines such as Thrombopoietin (TPO), Angiopoietins (Angs), and wingless-related integration (Wnt), which involve in the survival, quiescence, and self-renewal of HSCs [1]. MSCs produce growth factors that influence the maintenance of HSCs´ critical features in the

culture media. These cells additionally provide proper cell-cell contact and extracellular binding to support HSCs expansion [12].

Different blood cells differentiate from HSCs [13]. These mentioned cells produce common myeloid and common lymphoid progenitor cells [14]. HSCs could be divided into two categories based on their self-renewal ability: long-term hematopoietic stem cells (LT-HSCs) and short-term hematopoietic stem cells (ST-HSCs) [13].

LT-HSCs have high self-renewal capacity and are less affected by cytokines. ST-HSCs have low self-renewal capacity, are more active, and induce hemopoiesis for less time *in vitro* [13].

HSCs have markers, including CD34, expressed on all HSCs, and CD90; expressed on the primitive HSCs. On the other hand, the lack of CD38 marker on these cells indicates the early stages of hematopoiesis. Differentiation of HSCs into lymphoid and myeloid cells lead to expression of specific markers of each lineage [15].

HSCs status is affected by a specific microenvironment, called a niche, in the bone marrow. HSCs niche affects their fate by regulating their self-renewal, proliferation, and differentiation [15–17]. The HSCs niche is composed of various cells such as MSCs, osteoblasts, adipocytes, and fibroblasts [16].

HSCs are expanded in the laboratory using various methods for application in the therapeutic or research fields. Simulation of HSCs niche and utilization of MSC in HSC *ex vivo* co-culture, promotes HSC culturing condition and provides an adequate number of HSCs [18]

MSCs enhance the survival and self-renewal of HSCs by either secreting cytokines and growth factors or mediating direct cell-cell contact via adhesion molecules [2]. In other words, co-culturing of HSCs with MSCs mimics the natural hematopoietic microenvironment for HSC proliferation, self-renewal, and differentiation [19–21].

In recent studies using of bioreactors, scaffolds, nanoparticles, and nanofibrils are diverse practical methods to improve expansion conditions of HSCs *in vitro* [22, 23].

Co-culturing of MSCs with HSCs is commonly used for improving their proliferation. Numerous parameters such as cytokines, extracellular vehicles (EVs), scaffolding, three-dimensional culture, the source of MSCs, and hypoxia affect the co-culturing of these two cell types [17, 22]. The culture conditions of these cells could be strengthened by manipulating these parameters. In this article, we reviewed the impact of those mentioned parameters on HSCs expansion. It might suggest practical strategies to provide more competent cells for therapeutic purposes.

Cellular secretions

Studies indicate that MSCs produce and secrete many chemokines, cytokines, growth factors, and extracellular matrix molecules. Microenvironmental signals are generated in MSC's niche, that can regulate various physiological and pathological processes including cell proliferation, differentiation, tissue development, regeneration and tumorigenesis [24, 25]. Cytokines and hematopoietic growth factors such as SDF, GM-CSF, Angs [26, 27], as well as substances secreted from MSCs, including micro vesicles (MVs) and exosomes, are valuable tools, utilized *in vitro* and *in vivo* researches in terms of basic sciences and disease treatment [28, 29]

Cytokines

MSCs play a critical role in the maintenance and differentiation of HSCs in the bone marrow niche by secreting cytokines and growth factors [30]. Several cytokines involve in the HSC differentiation and proliferation including IL-6, FLT3L, SCF, GCSF, and GM-CSF [6]. These cytokines and hematopoietic growth factors are utilized in different HSC expansion methods to provide a sufficient number of HSCs [6]. Using of MSC-feeder layer results in more cytokine production in the culture media. In these conditions, differentiation into the myeloid lineage is more likely to occur as an effect of cytokines [31]. In a study, co-culture of bone marrow MSCs with cord blood HSCs with a cytokine cocktail of FLT3L, SCF, basic fibroblast growth factor (BFGF), and leukemia inhibitory factor (LIF) led to further differentiation into the myeloid lineage. At the same time, a significant number of cells maintained their potential for lymphoid differentiation [32]. Cytokines widely use in the *ex vivo* expansion of HSCs because of their role in regulating the fate of HSCs. For this purpose, by adding exogenous cytokines such as SCF, FLT3L, and TPO in the culture media in co-culture of MSCs with HSCs, more clonogenic cells could be proliferated and expanded [33]. High levels of SDF in the MSC-HSC co-culture system reduce the secretion of inhibitor cytokines that negatively influences the self-renewal capacity of HSCs and progenitor cells [34]. Ahmadnejad et al. examined the culture of HSCs in different conditions, culture with MSCs feeder layer, culture with MSCs feeder layer plus cytokine cocktail and cytokine cocktail only. They concluded that using MSCs feeder layer with cytokine cocktail enhances cell proliferation [35]. Furthermore, cell proliferation with cytokines only results in the low survival of CD34 + cells [36], highlighting the importance of using MSCs in co-culture with HSCs.

In another study, the usage of MSCs as feeder layer and inhibition of transforming growth factor-beta receptor II (TGFβ-RII) increased the number of $CD34^{+}$ cells, the total number of nucleated cells, and the number of colonies *in vitro* [37]. Therefore, using different cytokine cocktails in combination with MSC co-culture is one of the practical methods to optimize HSCs expansion conditions.

Microvesicles

Extracellular vesicles are cell-derived vesicles with a lipid bilayer membrane that ranges from 30 to 2000 nm based on their origin. Extracellular vesicles can be divided into three main classes: exosomes, micro vesicles, and apoptotic bodies, which differ in size and production mechanisms [38]. MVs establish

intracellular communication and they also interact with target cells by controlling their biological functions [39]. They contain cytokines, signaling proteins, microRNAs (miRNAs), and mRNAs of origin cells. They could affect subjected cells by delivering these mentioned materials [40]. MSC-derived MVs play an essential role in maintaining intra-tissue homeostasis and could control cell responses to different stimuli especially in disease or injury [41]. A study by De Luca et al., reported that MSC-derived MVs affect the gene expression pattern of umbilical cord blood $CD34^+$ cells and enhance their survival rate, inhibit their differentiation, and increase their migration to the bone marrow. miRNAs containing MVs interfere with the fate of umbilical cord blood $CD34^+$ cells. MSC-derived MVs cooperate in the regeneration of the hematopoietic compartments, which can be a suitable therapeutic tool for the treatment of various hematologic and non-hematologic diseases [42]. Figure 1 represents the effect of MSC-secreted cytokines and MSC MVs on HSCs briefly (Fig. 1).

Scaffold and 3-dimensional culture

Studies indicate the importance of a three-dimensional culture system and scaffolds for simulating the cells physiological condition *ex vivo* [43, 44]. Despite its easy application, a Two-dimensional culture system, has some limitations such as low cell proliferation capacity. In two- dimensional culture, a monolayer of cells is grown, contrary to the three-dimensional physiological condition of the tissue *in vivo*. Improper cellular interaction and risk of hydrodynamic damages are other potential limitations of two-dimensional culture conditions. The purpose of three-dimensional culture is increasing the surface-to-volume ratio, the contact surface and cell communication [45, 46]. Scaffolds act as a physical supporter, which increases the proliferation rate and then the number of expanded HSCs [43]. Typically, a two-dimensional layer of MSCs is used in the MSC and HSC co-culture to support $CD34^+$ cell proliferation. Two-dimensional culture does not adequately mimic bone marrow microenvironmental conditions and reduces the opportunities to cultivate engraftable HSCs with long-term culture ability [22, 47]. The three-dimensional culture of MSCs in a co-culture system not only increases their positive effects on the HSCs proliferation but also preserves HSCs self-renewal and stemness potentialities [48]. One of the strategies to strengthen MSCs and HSCs co-culture systems is the use of scaffolds to create a three-dimensional dynamic microenvironment [19]. Bioreactors, nanoparticles, nanofibrils, and scaffolds could apply to improve HSCs cultivation conditions [49–51]. For example, in one study, fibronectin-coated polycaprolactone scaffolds were used to expand HSCs. The results showed an increase in $CD34^+$ cell number compared to the two-dimensional culture. Mousavi et al. isolated HSCs from the umbilical cord blood and cultured these cells in the presence of polycaprolactone nano scaffolds containing fibronectin. Cell enumeration results showed that the number of cells cultured with nano scaffold was much higher than the control group (culture under normal conditions without nano scaffold). Moreover, the expanded cells on the scaffolds had more clonogenicity [49, 50]. Futerga et al. reported that co-culture of MSC and HSCs in Poly dimethyl siloxane Microwells (PDMS) increased the number of $CD34^+$ $CD38^-$ cells, which was independent on MSCs but in response to PDMS properties [20]. In another study, MSC and HSC co-culture systems were established without using cytokines and other bioactive compounds in three-

dimensional culture with bone-made –scaffolds. The result showed significant increase of HSCs in this condition compared to two-dimensional culture [21].

Hypoxia

As mentioned earlier, HSCs locate in the bone marrow in particular microenvironments known as the HSC niche [16]. Stem cell niche has low oxygen pressure [40]. In physiological conditions, stem cells distribute in different tissues with low oxygen pressure [18]. The culture of mouse and human HSCs at 20% oxygen pressure causes stem cell depletion, while culture under 1% oxygen condition causes better cell survival [52]. The mechanism of HSC response to hypoxia conditions is probably related to hypoxia-inducible factor 1 (HIF-1), which activates at low oxygen pressure [53]. This situation changes the metabolic pathway from oxidative to glycolytic one, reduces the production of reactive oxygen species (ROSs), and enhances the degradation of these molecules. ROSs inhibit HSC differentiation [54]. Hypoxia seems to protect cells from oxidative stress damage [55]. Zhao et al. co-cultured Wharton jelly-derived MSCs with HSCs under 1% oxygen pressure (hypoxia) or 20% (normoxia), without the addition of external cytokines. Their results revealed that at 1% oxygen pressure, the number of $CD34^{+}$ Lin^{-} primary cells increased significantly [56]. 1% oxygen pressure reduces the differentiation and maintains the pluripotency of HSCs, while 3–5% oxygen pressure does not affect cell differentiation [57]. Mohammadali et al. found that mild hypoxia induces more rapid proliferation in HSCs and increases the number of expanded total nucleated cells. Moreover, expansion of human $CD34^{+}$/ $CD38^{-}$ cells under hypoxia conditions increases engraftment rate. Transplantation of hypoxia-expanded-HSCs leads to more reconstitution of hematopoietic tissue in mice *in vivo* [58].

Use of fetal-tissue-derived MSCs in co-culture with HSCs

Wharton jelly (WJ)-derived MSCs

WJ-MSCs as an easy-access source of MSCs have recently emphasized the attention of researchers due to their fewer ethical concerns. The population of WJ-MSCs multiplies faster than bone marrow-derived MSCs (BM-MSCs). These cells are more primitive in comparison with bone marrow MSCs and probably are also less immunogenic [59]. WJ-MSCs can be an ideal candidate for usage as feeder layer in a co-culture system due to their high proliferation capacity and rapid growth. WJ-MSCs express markers such as CD117 ([c-kit), and SCF receptors at a high level that helps to identify them in the co-culture with HSCs [60]. Osteopontin is highly expressed in WJ-MSCs [61]. On the other hand, WJ-MSCs can secrete hyaluronic acid [62]. Both of these molecules are significant constituents of HSC's niche. In particular, osteopontin is an essential regulator of HSC quiescence and proliferation. WJ-MSCs, like other MSCs, express CXCL12, which interacts with its receptor, CXCR4, to regulate HSC migration and homing. WJ-MSCs secrete most of the cytokines involved in the regulation of hematopoiesis. The concentration of

hematopoietic growth factors such as G-CSF and GM-CSF secreted by these cells is higher than the factors secreted by MSCs isolated from other sources such as bone marrow [63, 64]. Then it seems that the application of WJ-MSCs as feeder layer might improve the expansion condition of HSCs.

Umbilical cord/ Umbilical cord blood-derived MSCs

BM-MSCs isolation is an invasive and painful procedure. But MSCs can isolate from embryo associated tissue, such as the umbilical cord (UC) or umbilical cord blood (UCB). UCB and UC are rich sources of MSCs and be considered for isolation of these valuable cells [65] UC-MSCs secrete more than 200 biologically active molecules, including cytokines, chemokines, and hematopoietic growth factors [66]. Therefore, UC used as an alternative source of MSC for clinical applications [67]. UC-MSCs are more initial/ primary than BM-MSCs. In addition, UC-MSCs proliferate faster than BM-MSCs. In the study of Wu et al., a sufficient number of UC-MSCs obtained for transplantation within eight days, which is shorter than the time required for BM-MSC harvesting [68]. UC-MSCs do not express HLA class II molecules on their surface. But they express B7-H1, a negative regulator of the immune system. Then they also prevent the reaction and activation of T lymphocytes and dendritic cells [69]. They also release indoleamine 2,3-dioxygenase and IL-12 suppress the immune system and increase graft survival [70]. These cells enhance the proliferation of regulatory T lymphocytes and are effective in treating graft versus host disease [71]. UC-MSCs and UCB-MSCs secrete SDF-1, Flt3, TPO, G-CSF, GM-CSF, IL6, SCF, and LIF, which play a vital role in supporting HSC growth. Gao et al. found that UC-MSCs promote HSC growth rate and elevate the expansion of megakaryocyte colony-forming units (CFU-Mk) even more efficiently than BM-MSCs [72]. The use of embryonic-tissue- derived-MSCs in the co-culture system with the HSCs can increase the efficiency of the HSCs expansion method and provide a sufficient number of the cells for transplantation. Examples of co-culture invigorating factors and their mechanisms have been summarized in Table 1.

Table 1
Use of scaffolds, hypoxia, and umbilical cord/ umbilical cord blood-derived MSCs to strengthen MSC and HSC co-culture system and their effect.

Factors		Mechanism	Effect	Reference
Scaffold	PCL scaffold coated with FN	↑ CXCR-4, VLA-4, VLA-5, LFA-1, HOXB-4, HOXA-9, BMI-1, Htert ↑CD13, CD14, CD33, CD34, CD45 ↓CD2, CD3, CD19	↑Total number cells ↑Proliferation ↑Adhesion ↑Homing ↑Self-renewal	[49, 50]
	Polydimethylsiloxane microwells in the PDMS	↑CD34, ↓CD38	↑Homing	[20]
	Biomimetic macroparous PEG hydrogels 3D scaffold	Mimicking the HSC niche	↑Stemness	[51]
Hypoxia	Hypoxia tension 1% O_2	↑ VEGF, ↓ IL-6, ↓IL-7, ↓ SCF, ↓TPO of WJ-MSCs Activation of NOTCH, Wnt/βCatenin, Hedgehog signaling pathway	↑Total number cells ↑Stemness ↑CFUs ↑$CD34^{+}Lin^{-}$ Cells	[56]
	MSC feeder + cytokines and 5% O_2 tension	↑CD34 ↑ CXCR4 gene ↑ CXCR4 mRNA	↑Total number cells ↑Homig ↑CFUs ↑Migration	[58]
Embryo-dependent-tissue-derived MSCs	UCB-derived MSCs	↑ CD34 ↑ TPO ↓ GM-CSF, SCF	↑Proliferation of $CD34^{+}$cells, ↑CFU-GM, ↑CFU-E ↑CFU-GM	[72]
	WJ-derived MSCs	↑CD34	↑HSC expansion	[64]

Use of chemicals to strengthen MSC and HSC co-culture system

In most cases, the *ex vivo* culture of HSCs requires the addition of cytokines. However, cytokines often lead to the accumulation of ROSs in the HSCs. Accumulation of ROSs causes excessive oxidation of nucleic acids, lipids, amino acids, and carbohydrates. It not only induces senescence and apoptosis in the $CD34^+$ population but also leads to carcinogenesis and impaired hematopoiesis [73, 74]. Then providing some other HSC expansion stimulators might be effective in this regard.

Chemical and herbal molecules use to improve the co-culture conditions of HSCs and MSCs. Resveratrol is a natural polyphenol found in various plants and fruits, including peanuts, berries, and grapes [75]. Resveratrol has various functions such as reducing oxidative stress, influencing the cell cycle, and binding to receptors on the HSCs surface. Therefore, it considers as a culture medium additive, which increases HSCs proliferation and improves their cultivation conditions [76].

Tang et al., proved that the addition of 10 μM resveratrol can induce $CD34^+$ cell proliferation *ex vivo*, maintaining primary HSCs ($CD34^+$/ $CD38^-$/ $CD45R^-$/ $CD49f^-$/ $CD90^+$ cells), and inhibits their apoptosis [77]. 1 μM resveratrol reduces ROSs and nitric oxide levels in HSCs. It might more effective in the co-culture of HSCs with MSCs and protects HSCs against apoptosis [78]. Copper involves in gene expression regulation, protein function, and cell proliferation and differentiation. However, it catalyzes ROSs production. Then an excessive amount of intercellular copper induces harsh toxic conditions. Zaker et al. showed that the addition of TEPA, a linear polyamine copper chelator, to the culture medium of HSC and MSC co-culture; while reducing the amount of intercellular copper, induces more proliferation of $CD34^+$ cells compared to other culture conditions. TEPA preserves HSCs storage pool in bone marrow by reducing oxidative stress [79]. Zhang et al. studied the use of some chemical components and small molecules to increase HSC proliferation. In this investigation, it was shown that various chemicals including SR1 (aryl hydrocarbon receptor antagonist), UM171 (pyrimidoindole derivatives), P18IN003 ,and P18IN011 (p18 inhibitory molecules in the cell cycle), CHIR99021 (type of glycogen synthase kinase 3β inhibitor), rapamycin (mTOR inhibitor), BIO (another kind of glycogen synthase kinase 3β inhibitor), NR-101 (a small non-peptidyl agonist molecule of c-MPL), 5azaD / TSA (5-aza-2-deoxycytidine / trichostatin A), and GAR (garcinol; a plant-derived histone acetyltransferase) could increase HSC self-renewal capacity [80]. Both VPA (Valproic acid) and DEAB (diethylaminobenzaldehyde) by preventing HSCs differentiation and zVADfmk / zLLYfmk (caspase and calpain inhibitors) and 5-HT (5hydroxytryptamine or serotonin) by inhibiting HSC apoptosis enhance HSCs proliferation [80]. Adding these materials to the culture medium of HSC and MSC co-culture might be effectively optimizing the HSC cultivation condition and expansion. Table 2 represents mechanisms and effects of using chemicals in MSC and HSC co–culture system that has reported in different studies.

Table 2
Using chemical and herbal molecules to strengthen MSC and HSC co–culture system and their effects.

Kind of Chemical/ Component	Mechanism	Effect	References
Resveratrol	↓ Intracellular ROS level; ↑Strengthening the scavenging of ROS ↓Percentages of apoptotic cells in cultures	↑ Expansion of $CD34^+$ cells Preserved more primitive HSCs ↑Biological function	[76–78]
TEPA	↑ Cellular Copper content	↑ HSC Expansion	[79]
	↓ Oxidative stress	HSC storage pool preservation	
SR1	↓ AhR	↑ Number of $CD34^+$ cells	[81]
UM171	↑Genes encoding surface molecules [TMEM183A and PROCR]	↑ Human HSC self-renewal	[82]
P18IN003 P18IN011	↓ $p18^{INK4C}$	↑The frequency of primitive hematopoietic cells culture	[83]
CHIR99021 Rapamycin	↓ mTOR ↑Wnt/β-catenin and PTEN/PI3K/AKT/mTOR	↑ HSCs ↑ Cell cycle progression	[84, 85]
BIO	↓ GSK3β	↑ HSC self-renewal	[86]
NR-101	STAT5 activation	↑ Human HSC self-renewal	[80]
	HIF-1α stabilization	Regulate the oxygen level Response to hypoxia ↑ Expression of VEGF	
5azaD TSA	↓ DNA methyltransferase	↑ HSC self-renewal	[87]
GAR	↓ HAT	↑ HSC self-renewal	[88]
VPA	↓ HDAC	↓ HSC differentiation	[89]
DEAB	↓ ALDH ↓ Expression of cEBPε and HoxB4	↓ HSC differentiation	[90]
5-HT	5-HTR activation ↓ Caspase-3 level	↓ HSC apoptosis	[91]

Kind of Chemical/ Component	Mechanism	Effect	References
zVADfmk zLLYfmk	↓ Caspase activity ↑ Bcl-2 ↓ Caspase-3 Notch1 activation	↓ HSC apoptosis	[92, 93]

ROS: Reactive oxygen species. AhR: Aryl hydrocarbon receptor. TMEM183A: Transmembrane protein 183A. PROCR: Protein C receptor. mTOR: Mammalian target rapamycin. PTEN: Phosphatase and tensin homolog. PI3K: Phosphoinositide 3-kinase. GSK3β: Glycogen synthase kinase 3 beta. STAT5: Signal transducer and activator of transcription 5. HIF-1α: hypoxia inducible factor-1α. 5azaD: 5-Aza-2-Deoxycytidine. TSA: Trichostatin A.HAT: Histone acetyltransferase. HDAC: Histone deacetylase. DEAB: Diethylaminobenzaldehyde. ALDH: Aldehyde dehydrogenase. VPA: Valproic acid. GAR: Garnicol. 5-HT: 5-Hydroxytryptamine.

Non-adherent suspension cultivation

MSCs typically proliferate as monolayer adhere cells due to their intrinsic nature [5, 19]. Cell cultivation in the adherent phase could also cause some disadvantages and limitations. After a while, adherent culture in a monolayer phase led to decreased cell proliferation and expansion capacity [21]. The differentiation capacity and cellular plasticity of MSCs reduce during adhesive culture and multiple cell culture passages. The secretory capacity and the amount/ type of bioactive molecules secreted by MSCs often change and decrease gradually under adhesive conditions. On the other hand, the rate of cellular senescence increases in monolayer and adhesive culture [21, 24].

Non-adherent culture for colony/sphere formation is one of the practical methods to improve the differentiation and secretory capacities of MSCs [18, 25, 26]. In this case, cells proliferate in colony, and cell-cell contact increase during the culture period time. Then cell communication and signaling are performed effectively. The suspension culture of MSCs enhances their protective effects on HSCs by increasing paracrine properties [27].

MSC Non-adherent culture creates partial three-dimensional and dynamic culture condition and improves their proliferation, paracrine, differentiation abilities [26]. According to evidence, suspension cultivation of MSCs and MSC-spheres could be used as a simple method to enhance the efficacy of the co-culture system [18].

Discussion And Conclusion

Nowadays, the use of MSCs and their derivatives have been considered in cell therapy fields to treat various diseases [57, 94]. The features of MSCs including, secretion of cytokines, migration/tropism to the injured tissues, immunomodulation, etc., have made them valuable tools for therapeutic approaches [95, 96]. HSC transplantation utilizes as a final treatment for autoimmune diseases, immunodeficiency, genetic disorder, hematopoietic tissue malignancies, and cancers [97]. Due to the therapeutic application of HSCs, the need for the effective expansion of these cells *in vitro* has increased [98]. Differentiation, proliferation, and stemness capacity of HSCs might reduce during their cultivation *in vitro* [47]. On the other hand, MSCs have supportive effects on HSCs and could use as a feeder layer [18]. Therefore, the co-culture of HSCs with MSCs is used to expand HSCs. By improving the co-culture system of these cells via examining the involved parameters, defining strategies to optimize it, and strengthening this culture method, we can harvest a sufficient number of competent cells for transplantation and other research uses. For this purpose, several studies have been conducted and different conditions of the co-culture have been investigated in terms of the presence of cytokines, use of scaffolds, adding various additives to the culture medium, modification on cultivation conditions, and oxygen pressure. Some of these conditions could be used in a combination manner [18]. These strategies were represented in Fig. 2.

Today, due to the therapeutic application of stem cells, especially HSCs, for the treatment of malignant and non-malignant diseases, the study of these cells has been considered *in vitro.* At the same time, *in vivo* studies have been designed to overcome the existing limitations. Indeed, the disadvantages and advantages of using these methods are not yet fully understood in the clinic. Therefore, more studies and researches should perform in this regard.

Abbreviations

FLT3: Fetal liver tyrosine kinase-3.

FGF: Fibroblast Growth Factor.

HSC: Hematopoietic Stem Cell.

MV: Micro vesicle.

SCF: Stem Cell Factor.

LIF: Leukemia Inhibitory Factor.

TPO: Thrombopoietin.

PCL: Polycaprolactone. FN: Fibronectin.

PDMS: Polydimethyl siloxane.

PEG: Polyethilene glycol.

CFU: Colony-Forming Unit.

CFU-GM: Colony-forming-unit granulocyte/macrophage.

CFU-E: Colony-forming unit erythroid.

IL: Interleukine.

VLA: Very late antigen.

LFA: Lymphocyte function-associated antigen.

GM-CSF: Granulocyte-macrophage colony stimulating factor.

VEGF: Vascular endothelial growth factor.

UCB: Umbilical cord blood.

MSCs: Mesenchymal stem cell.

WJ: Wharton jelly.

HSC: Hematopoietic stem cell.

ROS: Reactive oxygen species.

TEPA: Tetraethylenepentamine.

AhR: Aryl hydrocarbon receptor.

TMEM183A: Transmembrane protein 183A.

PROCR: Protein C receptor.

mTOR: Mammalian target rapamycin.

PTEN: Phosphatase and tensin homolog.

PI3K: Phosphoinositide 3-kinase.

GSK3β: Glycogen synthase kinase 3 beta.

STAT5: Signal transducer and activator of transcription 5.

HIF-1α: hypoxia inducible factor-1α.

5azaD: 5-Aza-2-Deoxycytidine.

TSA: Trichostatin A.

GAR: Garnicol.

HAT: Histone acetyltransferase.

VPA: Valproic acid.

HDAC: Histone deacetylase.

DEAB: Diethylaminobenzaldehyde.

ALDH: Aldehyde dehydrogenase.

5-HT: 5-Hydroxytryptamine.

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Mandana Shirdarreh has written the first draft of the manuscript. Mohammad Pouya Samiee and Armita Safari have participated to write the first manuscript and figure preparation. Fatemeh Amiri has reviewed the text. All authors commented on previous versions of the manuscript. All authors reviewed the manuscript.

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Figures

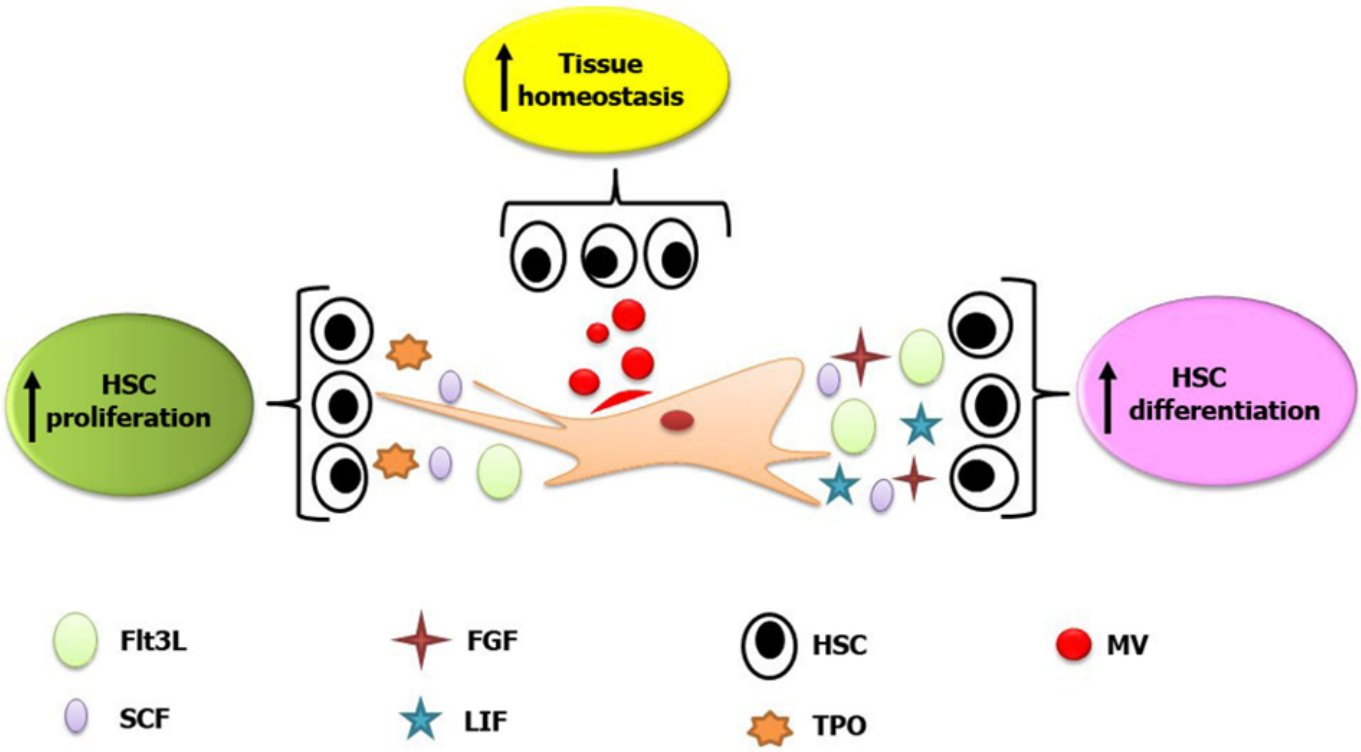

Figure 1

Cytokines and MVs effect in MSC-HSC co-culture. FLT3: FMS-like tyrosine kinase-3-ligand. FGF: Fibroblast Growth Factor. HSC: Hematopoietic Stem Cell. MV: Micro vesicles. SCF: Stem Cell Factor LIF: Leukemia Inhibitory Factor. TPO: Thrombopoietin.

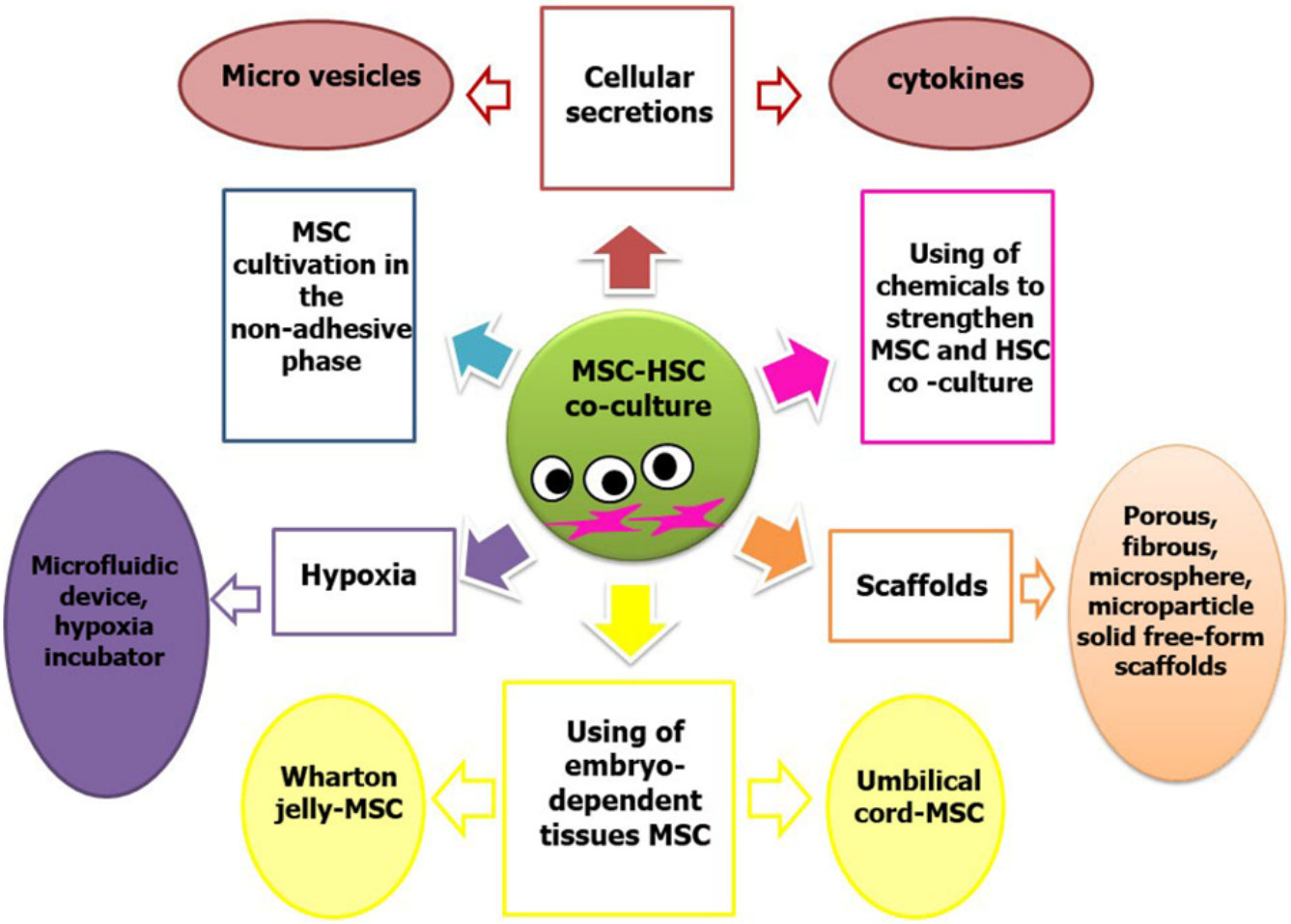

Figure 2

Several influential parameters in MSC-HSC co-culture. MSCs: mesenchymal stem cell. HSC: Hematopoietic Stem Cell.