Non-gynaecological applications of menstrual-derived stem cells: a systematic review

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Abstract

Menstrual-derived stem cells (MenSC) are a potential novel source of mesenchymal stem cells. There is an increased interest in investigating the therapeutic potential of MenSC due to the

various advantages they exhibit, when compared to other types of stem cells. MenSC are obtained non-invasively from menstrual blood. Thus, collection of MenSC is simple, reproducible and can be carried out periodically, with minimal complications. MenSC are present in abundance, are highly proliferative, exhibit a low immunogenicity and lack ethical issues. MenSC have shown the ability to differentiate into several lineages. The therapeutic potential of MenSC in non-gynaecological applications has been investigated in wound healing, neurological, musculo-skeletal, cardiovascular, respiratory, and liver disorders, as well as in diabetes and cancer. Human clinical trials are limited. To date, therapeutic efficacy and safety have been reported in patients with Avian influenza A subtype H7N9, COVID-19, congestive heart failure, multiple sclerosis and Duchene muscular dystrophy. However, further clinical trials in humans should be conducted, to study the long-term therapeutic effects of these stem cells in various diseases and to further explore their mechanism of action. This systematic review focuses on the application of MenSC in non-gynaecological diseases.

Keywords: Menstruation, menstrual-derived stem cells, , mesenchymal stem cells, regenerative medicine, cell therapy.

Introduction

A novel source of mesenchymal stem cells was identified from menstrual blood in 2007 and they were called menstrual-derived stem cells (MenSC) [1,2]. Increased interest was shown

towards MenSC due to their abundance, continuous source, non-invasive collection via menstrual cups, low immunogenicity, high proliferation, differentiation ability and lack of ethical issues [3-5]. The mechanisms of action of MenSC are by differentiation into target cells, immunomodulation, paracrine cytokine secretion, migration and engrafting into injured sites [6]. MenSC can differentiate into several lineages including endothelial, osteogenic, chondrogenic, adipocytic, pancreatic, hepatic, respiratory epithelial, cardiomyocytic, and neurocytic lineages [1,7-10] (Table 1). MenSC have shown potential therapeutic application in various non-gynaecological disorders including wound healing [11-14], neurological [15-17], musculo-skeletal [18,19], cardiovascular [5,20,21], respiratory [5,20,22] and liver [23,24] diseases, as well as in diabetes [25,26] and cancer [27-31]. Several studies also considered the gynaecological applications of MenSC, such as in ovarian-related diseases [32,33], endometrial injury [34,35] and Asherman syndrome [36,37].

Meng et al. [38] were the first authors to report the presence of stem cells in menstrual blood which were referred to as endometrial regenerative cells. In the literature, various nomenclatures were used to refer to MenSC which are listed in Table 1.

Table 1: Nomenclatures used for MenSC (39)**.**

Other names used for MenSC	References
Endometrial regenerative cells	(38,40)
Endometrial stem cells	(41)
Endometrial mesenchymal stem cells	(42,43)
Menstrual blood-derived endometrial stem cells	(44,45)
Menstrual blood-derived mesenchymal stem cells	(46,47,48)
Menstrual blood-derived stem cells	(49-53)
Menstrual blood stem cells	(54)
Menstrual-derived stem cells	(55)
Menstrual stem cells	(56)
Menstrual blood progenitor cells	(57)
Menstrual blood stromal stem cells	(58-60)
Menstrual blood-derived stromal stem cells	(61,62)

To obtain MenSC, menstrual blood from healthy women is collected using menstrual cups. The exclusion criteria used in various studies were: vaginal infection history, non-steroidal anti-inflammatory drugs, corticosteroids and oral contraceptives use within the last 3 months, infections such as human immunodeficiency viruses, hepatitis C virus and hepatitis B virus, autoimmune diseases, diabetes, endometriosis and malignancies [63-66]. Menstrual blood for the isolation of MenSC should be collected on the day with the heaviest blood flow [65-66] usually the

second day [64,67-69]. Around 5 mL volume of blood will provide sufficient stem cells. The age range of the female participants in the studies ranged from 18 years to 45 years old with the most common age group being between 22 and 30 years [56,64-67].

The collected menstrual blood is transferred in phosphate buffered saline (PBS), amphotericin B, streptomycin, penicillin and ethylenediaminetetraacetic acid (EDTA) [38,44,56,70]. The temperature of the collected sample is maintained at 4ºC and is transported within 24 hours upon collection to the laboratory for processing[70]. The Ficoll-Paque density gradient centrifugation (DGC) method is used to separate menstrual blood mononuclear cells and then the separated cells are washed with PBS. The isolated cells from the middle white layer of endometrial cells are cultured in T25 flask containing modified Eagle's medium, 1% penicillin/streptomycin with high glucose, 1% amphotericin B, 15% foetal bovine serum (FBS) and 1% glutamine at 37ºC to obtain adherent cells [56,71]. The next day, the media are changed so that non-adherent cells are washed out. 0.05% trypsin-EDTA is used to detach adherent cells, the cells are then counted and sub-cultured. The culture media are changed twice a week and are incubated at 37ºC with 5% CO_2 and saturated humidity[71]. At 7 to 10 days of incubation when the cell confluency reaches 70%, the culture medium is removed and around 5mL of pre-warmed trypsin-EDTA is used to detach the cells for 2 to 3 minutes in CO_2 incubator. Trypsin is then neutralised by adding 10mL media containing 20% FBS and the cells are gently collected. The cells are passaged and, after 2 to 3 passages, there is sufficient cells for differentiation experiments [65,66]..

The DGC is the conventional method used for isolation of MenSC, however, in the buffy coat this method leaves flocculent membranes and karyocytes of the deciduous endometrium [38,58]. Most of the MenSC clones are produced by the deciduous endometrium. Moreover, it was noted that menstrual blood clots would interwind with deciduous endometrium after DGC. In the study by Sun et al.[72], a method for high yield isolation of MenSCs was tested which differs from the method described above. It was shown that MenSC remained in sedimentation after DGC. Red blood cell lysis buffer (RLBD) was used directly and compared to DGC. MenSC isolation was increased by RLBD. In this study, the DGC method was used but then from the buffy coat, the deciduous endometrium and karyocytes were transferred to a new tube and washed with PBS[72]. These were then suspended in high glucose DMEM medium and transferred into new tubes with supernatant removal. Then the red blood cell lysing buffer was added to suspend the sedimentation at room temperature for 3 to 5 minutes. RBC lysis was performed twice till most of the erythrocytes were lysed osmotically. Washing with PBS was

done and then the suspension of the sedimentation in growth medium was incubated at 37ºC with 5% humidified CO_2. This method collected a higher number of MenSC than the conventional DGC[72].

High concentrations of platelet rich plasma (PRP) affect MenSC culture duration. It was reported that the optimal PRP concentration for MenSC proliferation was 10% [73,74]. Processing of menstrual blood up to 72 hours after collection does not cause property changes in the MenSC plastic adherence [44]. For therapeutic application, MenSC must be expanded in culture so as to obtain enough cells for use. With increase of passage number, MenSC proliferation rate decreases gradually due to aging of MenSC[75]. There is a change in morphology in highly passaged cells as they increase in size, lose the fibroblastic morphology and appear senescent. High passing of stem cells (P20) causes gene alteration which are involved in stress response, transcriptional regulation, development, cell proliferation and apoptosis. Moreover, at high passages there is karyotype alteration. Zemel'ko et al. [76] reported that MenSC at 45 population doubling underwent aging. Meng et al. [38] showed that MenSC maintained normal karyotype with no tumour development after 68 PD. During MenSC passages, pluripotency protein expression of OCT-4 positive cells during the first passage was 97.5%, while when the twelfth passage was reached the positivity of OCT-4 positive cells was reduced to 19.4%. The study by Kahanmohammadi et al [51], it was reported that at the second and twelfth passage MenSC diploid phenotype was kept without any aberration in the chromosomes.

MenSC have the ability to differentiate into various lineages in the right conditions. Various studies have shown MenSCs ability to differentiate into osteogenic, adipocytes, chondrogenic, hepatocyte-like cells, germ-like cells, neural-like cells, cardiomyocytes and keratinocytes-like cells [38,56,77]. In most of the studies, prior to differentiation of MenSC into different lineage, the ability of MenSC to differentiate into adipogenic, chondrogenic and osteogenic was assessed.

MenSCs under light microscopy displays a spindle shaped and fibroblast-like morphology [38,78-80]. At low density platting, MenSC grow as large flat cells in a monolayer and fibroblast colony forming units are created. A spindled shaped fibroblastic morphology is observed when the cultured cells reached confluence. A homogenous cell population is noted upon approaching confluence. It is noted that this morphology persisted at later passages (P10)[79].

MenSCs express CD9, CD29, CD44, CD73, CD90, CD105 and CD166 cell markers. From these markers, CD9, CD29, CD73, CD90 and CD105 are commonly expressed in MSC. MenSC do not express CD14, CD34, CD38, CD45 and CD117 [81] nor haematopoietic stem cell markers such as CD34, CD45 and CD133 [38]. HLA-ABC was weakly expressed [81] while HLA-DR was not expressed indicating that MenSC have low immunogenicity [82]. Both endometrial MSC and MenSC co-express CD140b and CD146 [83-84] and these markers can be used to discriminate from endometrial stromal fibroblast ($CD146^{-}CD140b^{+}$) and endometrial endothelial cells ($CD146^{+}CD140b^{-}$)[85]. Human telomerase reverse transcriptase is expressed by MenSC [(38,51]. Borlongan et al. [86] reported expression of stage specific embryo antigen 4 (SSEA-4), homeobox protein NANOG and sex-determining region Y-box 2 (SOX2). It was noted that MenSC have different stem cell marker profiles which is dependent on the environment [86]. This might be a reason why expression of SSEA-4 varies from different studies ranging from 0 [87] to 19.4% [88]. It was also speculated that the difference in stem cell marker profile of MenSC can also be due to other donor's factors including age, contraception and environmental factors[77]. MenSC are commonly identified by the expression of octamer-binding transcription factor 4 (OCT-4) which an embryonic stem cell surface marker [38,51,56,58]

. OCT-4 is not expressed by endometrial MSC [89,90] or BMSC[91]. The molecule perivascular sushi domain containing-2 (SUSD2) specifies self-renewal and multipotency of MenSC[84].

In the studies by Meng et al. [38] and Wu et al.[57]it was reported that MenSC obtained from healthy and young women could increase in number, as every 20 hours it increases to one doubling under optimal culture conditions. MenSC doubling rate is twice faster than that of BMSC which is estimated at 40-45 hours [38,57]. MenSC have a high proliferation rate which is due to the elevated expression of extracellular matrix (ECM) and embryonic trophic factors [92]. For future research, the high proliferation ability of MenSC is important, as usually cell therapy is dose-dependent and low passage cells are used. Moreover, no obvious chromosomal abnormality was observed when MenSC were expanded *in vitro*. MenSC could have a huge clinical application due to their high proliferation rate, genetic stability and pluripotency [38,57,58].

Development of stem cells is associated to the Wnt signalling pathway. The Wnt signalling can regulate target genes that are downstream to mediate stem cell differentiation and proliferation[93,94]. Transcription in the Wnt signalling pathway is initiated by transcription factor β- catenin binding to the promoter site on the Wnt-responsive genes. Cytoplasmic β-catenin in

unstimulated cells is phosphorylated by glycogen synthase kinase 3 (GSK3). Inactivation of GSK3 occurs on binding of Wnt to Frizzled. Accumulation of cytoplasmic β-catenin occurs as a result of GSK3 inactivation and translocation to the nucleus to activate Wnt-responsive genes[94]. In a study by Kanzemnejad et al.[79], the role of Wnt signalling on MenSC proliferation was investigated. Different concentrations of lithium chloride (LiCl) were used, since LiCl decreases MenSC proliferation in a dose-dependent manner. The expression of β-catenin increased with increasing concentration of LiCl while MenSC proliferation was suppressed. This study shows that Wnt signalling pathway plays a significant role in MenSC proliferation. Further studies are still required to investigate the Wnt signalling in MenSC proliferation and differentiation into various lineages[79].

The aim of this systematic review was to evaluate the non-gynaecological applications of MenSC. A systematic search was performed within the electronic databases MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials, to identify all the articles on MenSC published in the English language from 2007 to August 2020, week 4 (see Appendix for the list of MeSH/EMTREE terms and keywords and the PRISMA flow diagram).

Method: A systematic search was performed within the electronic databases MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials, to identify all the articles on MenSC published in English language. The search was done from 2007 till August 2020, week 4, and used the MeSH/EMTREE terms and keywords reported in Tables 1, 2 and 3.

Results: The total number of articles retrieved were 1295: 584 from MEDLINE, 690 from EMBASE and 21 from Cochrane Central Register of Controlled Trials (Figure 1). Three additional relevant articles were retrieved from other sources: one was published in a journal not indexed in Medline (Ovid)/Embase/central, Chen et al.[39], the other 2 were published in February 2021, Xu et al. [62] and March 2021, Lu et al.[57]. Thus, they could not be retrieved from the databases search.

Clinical potential of MenSCs

The therapeutic potential of MenSCs has been investigated in both preclinical and clinical studies (Tables 3 and 4).

Wound healing

MenSC have shown the ability to differentiate into keratinocytes and epidermal layer cells which can overcome some of the limitations of keratinocytes isolation (such as low proliferation rate, time-consuming and difficult preparation procedures) [1,12,95]. Hence, MenSC could be clinically used for treatment of dermatological lesions and skin transplant [12]. MenSC improve chronic wound healing regeneration and repair through promotion of cell adherence, proliferation and differentiation [1,12,95]. MenSC overexpress the migratory molecule chemokine receptor type 4 (CXCR4) which binds to stromal cell-derived factor 1 (SDF-1) expressed by intrinsic fibroblasts, thus their migratory activity is directed to the site of injury [96]. MenSC showed increased fibroblast migration under both basal and pro-inflammatory conditions. The hypoxic agent deferoxamine stimulated higher expression of hypoxia-inducible factor (HIF)-1α in MenSC which displayed a higher angiogenic potential and increased factor expression (including platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF)) [97]. In turn, the migratory ability of fibroblast can be positively regulated by bFGF via the wnt/β catenin signalling pathway activation[98]. MenSC upregulated the genes involved in angiogenesis and neovascularisation, such as angiopoietin 1 (ANGPT1), PDGFA, PDGFB and matrix metallopeptidase (MMPS), extracellular matrix (ECM) components, elastin (ELN) and MMP10 that during remodelling of tissue helps to regulate collagen degradation[97]. MenSC showed a lower expression of serpin family E member 2 (SERPINE2), which is an inhibitor of serine protease with anti-angiogenic function, and lower expression of the pro-inflammatory interleukin (IL)-1β [97,99]. Transforming growth factor

(TGF)B2, a factor involved in keloid formation was downregulated in MenSC [97,100]. Table 3 explains the findings observed in wound regeneration *in vivo*.

Central nervous system disorders

In an *in vitro* model of Parkinson's disease (PD), when human neuroblastoma SH-SY5Y cells with neurotoxin 1-methyl-4-phenylprudunuym (MPP+) were co-cultured in a culture medium obtained from MenSC, there was a significant increase in injured cell viability which could be due to increased anti-inflammatory cytokine release. After treatment, a decrease of pro-inflammatory cytokines caused by neurotoxin, which include IL-1β, IL-6, inducible nitric oxide synthase (iNOS), cyclooxygenase (COX-2) and tumour necrosis factor (TNF)-α, was observed. The anti-apoptotic and antioxidant effects of MenSC culture medium could be caused by the presence of neurotropin (NT)-3, NT-4, brain-derived neurotrophic factor (BDNF) and epidermal growth factor (EGF) factors[101].

MenSC transplantation in a mice model of Alzheimer's disease (AD) showed amelioration of changes in the brain (Table 3). β-secretase (BACE1) and C-terminal fragment (β-CTF) levels, which are part of amyloidogenic pathway, were decreased. After MenSC treatment, the activated microglia were induced to express an alternative phenotype, instead of the neurotoxic phenotype characterised by anti-inflammatory cytokines secretions. IL-1β and TNF-α (pro-inflammatory factors) were significantly decreased while the anti-inflammatory cytokine IL-4 was increased. There was also increase in messenger ribonucleic acid (mRNA) levels of cluster of differentiation (CD)-206, YM1, arginase-1 (Arg1) and found in inflammatory zone 1 (Fizz1), which are markers of anti-inflammatory alternative activation of macrophages [15].

Upon culturing of MenSC with neurons in a rat stroke model, protection against ischaemia-induced cell death was observed. This could be due to elevation of trophic factors VEGF, BDNF

and NT-3 [102] which are reported to mediate therapeutic benefits of stem cell transplantation in various central nervous system (CNS) disorders [103,104]. In rat ischaemic model, motor and neurological impairment was improved (Table 3). MenSC survived in the ischaemic striatal penumbra and the cells were detected even after 14 days since MenSC transplantation. Graft survival was better when administration of MenSC was given intracranially (IC) rather than intravenously (IV) (15% survival rate for IC vs 1% for IV)[102].

For the first time, the study by Zhong et al. [16] demonstrated the feasibility of MenSC administration in 4 patients suffering from multiple sclerosis (MS) (Table 4). This study shows promise for future use of MenSC in clinical settings, as no immunoreaction or ectopic tissue were observed at the site of injection [16].

Rat with spinal cord injury treated with MenSC showed improved locomotor function, increased neuronal survival rate and preservation of tissue (Table 3). Neuronal survival rate increased in parallel with the increase of neuronal and glial cell markers neurofilament protein (NF)-200 and microtubule-associated protein 2 (MAP-2) significant increase[105].

MenSC transplantation with neural guidance conduits in a rat model showed significant improvement in the sciatic nerve injury. The reasons for this improvement might be due to the continuous secretion of neurotrophic factor and matrix protein by cells which assist in nerve repair[106]. MenSC induce angiogenesis which is essential for neural cell repair and functional recovery after nerve injury [8,107].

Musculo-skeletal disorders

MenSC have a chondrogenic differentiation potential, which translates in the expression of Collagen 9A1 and SRY-Box Transcription Factor 9 (SOX9) at mRNA level, similarly to cartilage tissue [108]. Insulin-like growth factor-binding protein 3 (IGFBP3) lacking MenSC were

able to differentiate into chondrogenic lineage which could be due to higher sensitivity to the chondrogenic promotor factor, insulin growth factor 1 (IGF-1)109. Moreover, improvement was observed in osteochondral defect *in vivo* (Table 3)[110].

In mice models of Duchenne muscular dystrophy (DMD), MenSC exhibited extensive migratory ability and infiltration between muscular fibres. MenSC exhibited a myoblast differentiation, which could be an important finding for the future treatment of muscular diseases. Exposure of MenSC to 5-azacytidine provides the highest desmin positive MenSC. MenSC do not express the genes myogenic factor 5 (Myf5) and myosin heavy chain IIx/D (MyHC-IIx/d); however, the genes desmin, myogenic differentiation protein (MyoD) and dystrophin were expressed. MenSC administration improved dystrophin delivery and muscle regeneration [18]. On the contrary, in the study by Ay et al. [111]MenSC seeded on synthetic scaffold did not exhibit spontaneous differentiation and did not attach to the synthetic fibres [111]. MenSC have been tested on a human individual with DMD and clinical improvement was observed and maintained for at least 2 years (Table 4)[112].

MenSC, when cocultured with nucleus pulposus cells, show higher cell density and secretion of collagen II and aggrecan, which are important in functional maintenance of the intervertebral disc. Keratin 19 (KRT19), carbonic anhydrase 12 (CA12) and forkhead box F1 (FOXF1) genes are markers for nucleus pulposus and they are significantly enhanced in co-culture with MenSC. This indicates that MenSC may have possible repair potential on nucleus pulposus[113].

MenSC injected in mouse limb ischaemia models at the site of ischemia shows significant improvement in the ischaemic area, especially when compared to untreated mice (Table 3). The best results have been obtained in the group of mice that were injected and infused with MenSC. Intramuscularly (IM) injected mice showed better improvement than IV injected mice. MenSC transplantation, due to the factors secreted (VEGF, BDNF, NT-3) aid in the formation of small

vessels in critical limb ischaemia (CLI) mice [19]. There is an ongoing human phase I/II clinical trial of MenSC in CLI patients[114].

MenSC, when cocultured with Achilles tendon cells, show the ability to differentiate into tenogenic cells. The differentiated MenSC express the genes for collagen I and collagen III which are important for maintenance of tendon function. The Achilles tendon markers thrombospondin-4 (THBS4), tenascin C (TENC) and scleraxis (Scx) are expressed and ECM is produced which is similar to the origin of Achilles tendon[115].

Cardiovascular disorders

To mimic myocardial infarction, MenSC have been cultured *in vitro* with hydrogen peroxide (H_2O_2)-induced injured rat cardiomyocyte cells. An increased cell viability rate and inhibition on the rate of apoptosis was observed. The altered mitochondrial function observed in H_2O_2 treated cells was restored when these cells were cocultured with MenSC. MenSC coculture decreased the levels of pro-apoptotic proteins such as Bcl-2-associated X protein (Bax) and increased the levels of anti-apoptotic protein B-cell lymphoma (Bcl)-2 which was observed in H_2O_2 treated cells. Moreover, the migration ability of H_2O_2 treated H9c2 cardiomyocytes was increased when cocultured with MenSC, due to upregulation of N-cadherin [7]. *In vivo,* improved cardiac function has been reported (Table 3) due to high *in situ* cardiomyogenic transdifferentiation ability of MenSC, showing clear striation and sarcomeric α-actinin[116]. However, this is in contrast to the studies by Zhang et al. [20] and Jiang et al. [117]in which MenSC did not differentiate into cardiac lineage except for a small number of endothelial cells.

There is an ongoing clinical trial aiming to treat with MenSC 60 individuals with congestive heart failure (CHF). Preliminary data on 17 patients showed no serious adverse event [1181].

Another study reported a 74-year-old patient with CHF who received 5 shots of MenSC and cord blood via IV in a period of 7 months (Table 4)[119,].

Respiratory system disorders

MenSC and MenSC derived exosomes have been administered to bleomycin (BLM)-induced mice model and pulmonary fibrosis improved, as alveolar epithelial cell apoptosis were regulated [120,1214]. MenSC were principally captured and absorbed in the lungs when injected through the tail vein [120,122]. The mice model showed significant improvement in pulmonary fibrosis (Table 3) [120-122]. Zhao et al.[121] reported that MenSC acted on the microenvironment of fibrosis with increase of anti-fibrotic factors, such as hepatocyte growth factor (HGF) and MMP-9. The apoptotic factor Bax expression is reduced in lung tissue after MenSC treatment which might offer protection to alveolar epithelium cells from apoptotic damage. Secretion of TGF-β, which has a critical role in the changes observed in interstitial lung disease, has been shown to be reduced after MenSC treatment, thus potentially explaining why pulmonary fibrosis is alleviated after treatment[121].

In vivo and *in vitro* results in acute lung injury (ALI) show that MenSC can migrate and stay in the injured area. *In vivo* improvement of lung function even at histological level was reported (Table 3). MenSC regulate the expression of cytokines to attenuate the inflammatory response[122,123]. The mitogen keratinocyte growth factor (KGF) expression increased after treatment with MenSC[123]. The proliferative index has increased while caspase 3, an apoptotic index was reduced after treatment. Levels of proteins Phosphoinositide 3-kinase (PI3K), β-catenin and vascular endothelial (VE)-cadherin were increased after MenSC treatment, while downregulation of phospho glycogen synthase kinase 3 beta (p-gsk3β), p-β-catenin and p-src

was observed. The increase in VE-cadherin and β-catenin suggests that pulmonary microvascular permeability is improved after MenSC transplantation[122].

A clinical trial on humans with H7N9 infection was performed and even after 5 years from therapy amelioration of symptoms related to H7N9 infection have been reported (Table 4). However, the evaluation on the long-term effects of MenSC on all patients was not possible as some refused follow-up, which is a limitation of the study. The first results of a clinical trial conducted on 26 COVID-19 patients treated with MenSC (ChiCTR2000029606) [123] have reported amelioration of clinical manifestations (Table 4)[124]. MenSC have already shown therapeutic potential in H7N9 patients who share similar clinical manifestation to COVID-19 patients [125].

Gastrointestinal disorders

MenSC treatment in animal models of ulcerative colitis has led to an amelioration of clinical symptoms, signs and histological alterations (Table 3). There is a reduction in macrophages-1 positive cell infiltration and intra-colon neutrophils in MenSC treated mice. The anti-inflammatory IL-4 and IL-10 were upregulated, while the pro-inflammatory cytokines IL-2 and TNF-α were downregulated. $CD11c^+MHC\text{-}II^+$ DC were decreased in MenSC treatment, which indicates that MenSC exhibit an immunomodulatory effect in controlling colitis development. $CD3^+CD25^+$ active T cells have also been downregulated. In mice treated with MenSC there was an increase of $CD4^+CD25^+Foxp3^+$ Tregs, which is related to immune tolerance maintenance and autoimmune reaction downregulation, leading to reduction of colitis. $CD3^+CD8^+$ T cell levels were reduced, which improves cytotoxicity and immune system dysfunction [126]. Programmed death-ligand 1 (PD-L1) which is important in immune suppression was expressed on MenSC cell surface and increased with stimulating factor concentration. This has contributed to reduced proliferation of inflammatory cells and effector

function. It was reported that blockage of PD-L1 reduced MenSC efficacy in colitis treatment, since MenSC require PD-L1 for colitis attenuation. Inflammation in colitis is reduced by MenSC via PD-L1, by reducing neutrophils, $CD3^+$ T cells and increasing alternatively activated macrophages [127].

In animal models of liver fibrosis, MenSC have shown migratory ability into the liver lobule [128]. The liver function tests have improved after transplantation (Table 3). In fact, there was rapid improvement of liver regeneration which indicate that the main mechanisms of liver repair by MenSC is unlikely to be by homing or by trans-differentiation. Instead, the likely mechanism of action is by trophic effect for hepatic tissue regeneration. Efficiency in liver regeneration by MenSC was the same in male and female mice models, indicating no gender difference in treatment outcomes, unlike other types of stem cells [129]. MenSC can reduce lymphocyte antigen 6 complex locus G6D (Ly6G) positive cells in the liver. This gives an indication that MenSC act by suppressing infiltration of inflammatory cells to alleviate damage. MenSC was able to inhibit monocytes differentiation into dendritic cells to control liver injury. T cell accumulation is also inhibited as MenSC shows a reduced expression of $CD4^+$ and $CD8^+$ T cells [128]. miR-122 hepatic levels correlate with the severity of liver damage and are downregulated after MenSC treatment [130]. LX-2 human hepatic stellate cell proliferation is suppressed by MenSC, therefore indicating inhibition of stellate cells. There are increased levels of the anti-inflammatory monocyte chemoattractant protein-1 (MCP-1), while the secretion of proinflammatory IL-6 is reduced by MenSC. This indicates protective factor properties during the initial stages of fibrogenesis. Although MenSC are able to ameliorate liver fibrosis, in order to reduce liver fibrosis there needs to be replenishing of hepatocytes 131. *In vitro* MenSC differentiate into hepatic lineage [10,132], however, *in vivo* only few cells differentiated. This could be due to the complex microenvironment [131]. Hepatocyte marker genes are present in liver-like cells derived from MenSC including albumin (ALB), cytochrome (CY) P450, CYP3A4, CYP1A1, CYP7A1,

alpha fetoprotein (AFP) and cytokeratin (CK)-19 [10,132,133]. Other detoxification enzymes expressed are glutathione S-transferase (GST)-A1, GSTA2 and GSTP1 [133].

Endocrine disorders

The use of MenSC *in vivo* in diabetic mice models shows MenSC distribution in various organs but mainly in the pancreas, which could be due to the signals secreted by the injured area. Symptoms and pancreas morphology in diabetic mice model were improved (Table 3). Upon testing there was no insulin or human C-peptide detected, which indicates that MenSC did not differentiate into insulin-producing cells in the injured pancreas [25]. MenSC were able to activate endocrine progenitor cells which reside in the duct, islet and exocrine tissue, and insulin increased indicating that MenSC promote the differentiation of pancreatic progenitor cells to β cells. Genes of β-cell development pancreas/duodenum homeobox protein 1 (pdx1) [25, 134], forkhead box protein A2 (foxa2), nkx6.1, neurogenin-3 (ngn3), paired box 4 (pax4) and mafb were upregulated in MenSC treated mice which enhances β-cell generation. Genes associated with mature β-cell such as mafa, insulin and glucose transporter 2 (glut 2) were also upregulated [25].

Cancer

MenSC have been tested *in vivo* in animal models of different tumours, including glioma [30], squamous cell carcinoma (SCC) [28], hepatocellular carcinoma (HCC) [135] and prostate carcinoma [29] (Table 3). MenSC were able to migrate to the tumour location, even when infected with adenovirus AD35-sTRAIL [30]. It has been reported that MenSC interact with tumour cells via paracrine mechanisms [30, 135]. Tumour growth (weight and volume) was reduced after treatment [28,30,135]. In HCC the proliferation rate of the tumour was reduced. Tet methylcytosine

dioxygenase (TET)-1 and TET2 expression was increased in hepatocyte cells after coculture with MenSC. This indicates that MenSC regulate DNA methylation via various methylation enzymes. MenSC can regulate epigenetic mechanisms found in HCC cells [135]. Inhibition of angiogenesis was observed in prostate carcinoma [29] and SCC [28]. In prostate tumour, after MenSC treatment, levels of VEGF and NF-κB activity were reduced [29].

Discussion

An increased interest on these types of cells was shown by researchers due to the various advantages MenSC exhibit. MenSC are procured by an easy non-invasive method, they are abundant and a continuous source, they have low immunogenicity, high proliferation rate, differentiation ability into different lineages and lack of ethical issue[77]. They can be obtained periodically and this is crucial for clinical efficiency. Sources such as bone marrow are obtained invasively and their number of culture passages are limited until the cells lose their stem cell potential and therefore MenSC are better in this aspect. Periodic collection can produce higher therapeutic dosages from the same genetical background. In addition, there are other functional properties of MenSC which can underlie specific uses. MenSC have a high proliferation rate and are able to differentiate in multiple cell lineages [56,77]. Furthermore, MenSC have the ability to expand without losing the normality of the karyotype or tumorigenic potential up to 68 doubling [38,58]. MenSC therapeutic potential has been studied in various areas both *in vitro* and *in vivo* (animal models and humans). However, although no adverse events were reported after MenSC treatment in humans and therapeutic effects were observed in a study on multiple sclerosis [136] and AS[61], more studies are required to evaluate any challenges prior to MenSC application in a routine setting.

There are also some limitations to use of MenSC that need to be acknowledged. MenSC have high rate of proliferation, however, time is required for the cells to multiply and achieve quantities which are sufficient for therapeutic applications. Collection of MenSC can be obtained only from pre-menopausal women; however, also males and post-menopausal women are prone to diseases such as stroke and neurodegenerative disorders. This problem could be solved by educating females on the potential therapeutic applications of menstrual cells and by offering harvesting and cryopreservation to pre-menopausal women for future autologous use. Allogenic menstrual blood can be used to treat males and even post-menopausal women[43].

Although MenSC exhibit some limitations, they also exhibit various advantages which may outweigh these limitations. They have shown a potential in future therapy in various areas. It is important that further studies, especially in humans, do not just evaluate the long-term safety of MenSC but also the effects of MenSC on various diseases. Moreover, establishing a protocol that maximises the therapeutic potential of MenSC with limited adverse reactions is also crucial[137]. It is important that a standardised protocol is devised for MenSC according to the diseases and also to the different age groups that might receive this treatment. Various routes of MenSC administration have been investigated and there is the need for studies which focus on the best method to transplant MenSC to achieve maximal therapeutic benefit according to the disorder. MenSC have shown therapeutic effects in various diseases, however, the underlying mechanisms and signal pathways are still unknown [39, 138]. Although more work is required on MenSC, they have shown multi-functional roles in the treatment of various diseases in preclinical research which paves the way for further development of MenSC therapy in clinical and regenerative medicine [39].

Conclusion

MenSC have shown a very promising therapeutic effect in various diseases. Studies conducted in humans with H7N9, COVID-19, CHF, MS and DMD reported positive therapeutic effects without any adverse events. However, more studies are required to evaluate any challenges prior to MenSC application in a routine setting. Further research on MenSCs is required regarding age of donor, transplantation routes, appropriate dose, eligible conditions, long-term monitoring and mechanisms of action. It is important that a standardised protocol is devised for MenSC according to the diseases and to the different age groups that might receive this treatment. No long-term data on MenSC exist and therefore more studies should focus on this aspect to examine their long-term safety and the survival time of MenSC. Although more work is required on MenSC, they have shown multi-functional roles in the treatment of various diseases in preclinical research which paves the way for further development of MenSC therapy

in clinical and regenerative medicine. The ease of rapidly obtaining MenSC in large amounts makes them unique, thus sperheading their application in clinical practice.

Table 2: Cellular differentiation ability of menstrual-derived stem cells.

MenSC differentiation	Contents of the culture medium	Expression of markers that confirm differentiation
Osteogenic differentiation	Dexamethasone, ascorbate, glutamine, penicillin-streptomycin, β-glycerophosphate and FBS [7]	OSTF1 [139]
Adipogenic differentiation	Adipogenic medium	Adipogenic markers; PPAR-γ, LEPR and LPL [140]
Chondrogenic differentiation	Insulin-transferrin-selenium, DMEM-HG, streptomycin/penicillin, dexamethasone, ascorbic acid-2 phosphate, sodium pyruvate and TGF-β1 [8]	Chondrogenic markers; IGF-1, FGF2, Activin and Collagen 2 protein [141]
Keratinocyte differentiation	MenSC co-cultured with keratinocytes derived from foreskin	Keratinocyte markers; keratin 14, p63 and involucrin IVL [95]
Neural-like cell differentiation	Serum-free P4-8F medium fortified with FGF-2 and EGF. For induction of terminal neural differentiation the culture is composed of neurobasal medium with BDNF, FBS, horse serum, nitrogen supplement, penicillin/ streptomycin and all-trans retinoic acid [9]	Nestin, GFAP, MAP2, GABBR-1, GABBR2 and TUBB3 [9,142]
Cardiomyocyte-like cell differentiation	DMEM containing 5-azacytidine and bFGF	Connexin 43 and troponin T expression [21]
Hepatocyte-like cell differentiation	Media supplemented with dexamethasone, insulin, transferrin, selenium, NTA and HGF were used initially to induce differentiation. Medium used for maturation contains FBS, DEXA, ITS+1 and OSM	Albumin and CK-18 [10]

Legend: bFGF: basic fibroblast growth factor, CK-18: cytokeratin 18, DMEM-HG: dulbecco's modified Eagle's high glucose, FBS: foetal bovine serum, FGF2: fibroblast growth factor-2, GABBR: gamma-aminobutyric acid type B receptor, GFAP: glial fibrillary acid protein, HGF: hepatocyte growth factor, IGF-1: insulin-like growth factor 1, ITS+1: insulin, transferrin, selenium pre-mix, LEPR: leptin receptor, LPL: lipoprotein lipase, MAP2: microtubule-associated protein 2, MenSC: menstrual derived stem cells, OSM; oncostatin M, OSTF1: osteoclast-

stimulating factor-1, PPAR-γ: peroxisome proliferator-activated receptor gamma, TGF-β1: transforming growth factor beta 1, TUBB3: tubulin beta 3 class III.

Table 3: Preclinical animal studies involving menstrual-derived stem cells in non-gynaecological diseases.

Disease		Animal model and route of administration	Findings after MenSC treatment
Wound healing		C57bI/6 mice, injected intradermal around each wound [97]	• Accelerated wound healing due to production of adhesion molecules ICAM-1 and VEGF • Increased vascular network formation with increased expression of pro-angiogenic factors VEGF, IL-8 • High collagen deposition with gene upregulation of elastin, fibronectin, collagen and MMP • Presence of MenSC at site of injury for more than 2 weeks after skin transplantation
Central nervous system disorders	**Alzheimer's disease**	APPswe/PSEN1dE9 mice injected to hippocampus [15]	• Improved spatial learning and target-oriented swimming pattern • Reduced amyloid β plaque deposition • Improvement of microglia activation • Improvement of Tau hyperphosphorylation through inactivation of GSK-3β
	Stroke	Rat stroke model, administered via IC or IV [102]	• No tumours or ectopic formations and no graft-versus-host complications • Reduced abnormal behaviour • Better motor coordination with IC injection • In the striatal ischemic penumbra, there were more surviving host cells

	Spinal cord injury	Sprague-Dawley rats injected at the SCI site [105]	• Improved locomotor function • Improved tissue integrity due to reduced infiltration by cells of inflammation and reduced vacuolization • Reduced cavity formation • Neuronal cells survival rate increased at the site of injury • At the site of injury axonal regeneration was promoted • Secondary glial cell formation reduced • Pro-inflammatory factors supressed (IL-1β and TNF-α). BDNF expression enhanced
	Sciatic nerve regeneration	Rat model implanted with seeded neural guidance conduit [106]	• Prevention of muscle weight loss • Hot plate latency test was low • Sciatic nerve function improved
Musculo-skeletal disorders	**Osteochondral repair**	New Zealand rabbits, implantation of encapsulated MenSC in fibrin glue [110]	• No immune rejection • Defect after 3 months filled with Hyaline cartilage-like • Tissue well regenerated • Better amount of glycosaminoglycan
	Duchene muscular dystrophy	NOG mice or mdx-scid mice, injected intramuscular [18]	• Detection of MenSC between myocytes after 1 to 3 weeks from implantation. • Differentiation into myoblasts • Expression of human dystrophin in dystrophic mice

			• Improvement of muscle regeneration
	Limb ischaemia	Mouse hind limb ischaemia models. Injection at the site of ischemia or via the tail vein [19]	• Improvement of ischaemia and decrease in the degree of ischaemic damage • Muscle tissue functional and alive • Reduced tissue oedema and smooth blood vessels with cell survival improvement • Vasculogenesis and angiogenesis
Cardiovascular disorders	**Myocardial infarction**	• F344 nude rats [116] • Sprague-Dawley rats [20,120] • C57BL/6 (H-2^b), BALB/c (H-2^d), and C3H (H-2^k) mice [143]	• Improved cardiac function • Improved left ventricular fractional shortening • Improved ejection fraction • MI size reduced • Thickness of left ventricle increased • Collagen deposits reduced • Inhibition of the transition of endothelial to mesenchymal which contributes to tissue fibrosis progression
Respiratory disorders	**Interstitial lung disease**	BLM-induced C57BL/6J wild-type mice, injected via tail vein [120-122]	• Pulmonary fibrosis improved due to regulation of alveolar epithelial cell apoptosis and less collagen deposits • Anti-fibrotic factors HGF and MMP-9 were elevated • Reduced inflammation levels and pulmonary oedema • Decreased interstitial hyperplasia

			• Protective effect on pulmonary fibrosis due to reduction for fibre formation and promotion of recovery of lung fibrosis. • mtDNA damage, ROS and apoptosis were decreased which may have protective role on fibrosis and alveolar cell damage • Attenuation of oxidative stress
	Acute lung injury	ALI induced by lipopolysaccharides in mice model, transplanted IV [122,144]	• Alleviation of inflammation • increased the dry/wet ratio and mitigation of the thickened texture of the lung caused by the damage • Improvement of lung histopathology • Decreased oedema • Increase in sO2% and paO2/FiO2 ratio • Inflammation reduced • Improvement of pulmonary microvascular permeability
Gastrointestinal disorders	**Ulcerative colitis**	BALB/c mice model DSS-induced colitis injected IV [127,128]	• Less loss of body weight • Firmer stool and prevented bloody stool • Increased water and food consumption • Longer colon length and no bowel dilation • Ulceration almost healed

			• Relived oedema and mucosal hyperaemia • Improved structure of crypts and epithelium • Down regulation of autoimmune reaction and immune tolerance maintenance reducing colitis • Decreased inflammation
Liver	**Acute liver injury**	BALB/c mice [130,76,145] or C57BL/6 mice [129] injected intravenously	• ALT, AST, urea and total bilirubin were reduced • Rapid improvement of liver regeneration • alleviation of cytoplasmic vacuolization, infiltration of inflammatory cells and necrosis • Restore the recovery of glycogen storage • Improvement of histopathological appearance of the liver • Decrease deposition of collagen fibres in liver • Increased number of hepatocytes and parenchymal cells • Lesser necrotic areas • Lower cell apoptosis • Suppression of inflammation with alleviation of damage
	Liver fibrosis	C57BL/6 mice, IV injected [132]	• Reduction of collagen deposition • Liver function improved

			• Reduce liver fibrosis • Reduced inflammation
	Diabetes	C57BL/6 mice injected IV [25,26]	• MenSC located at islet structures, ductal and exocrine of the pancreas • Improved polyuria • Stable weight • Reduced hyperglycaemia • Improved insulin levels • Glucose tolerance improved • Improved survival rate • Higher islet size and β-cell number • Angiogenesis promotion • Enhanced re-epithelialization • Wound closure enhancement
Cancer	**Glioma**	Nude mice, IV [30]	• Infected MenSC were able to migrate to the glioma • Tumour cells decreased in viability and apoptosis increased by 20% after exposure to infected MenSC • Tumour size decreased, tumour growth inhibition and tumour apoptosis induced by TRAIL
	Hepatocellular carcinoma	Balb/c nude mice injected in tail vein [138]	• Reduced proliferation of tumour (Ki67 expression reduced)
	Prostate tumour	NOD mice [29]	• Angiogenic properties of the secretome derived from tumour cells were inhibited

	Oral squamous carcinoma	Syrian golden hamsters [28]	• SCC growth inhibition, with reduced tumour size and weight • Weaker angiogenesis with significant reduction in vascular area and vessel density

Legend: ALI: acute lung injury, ALT: alanine aminotransferase, AST: aspartate aminotransferase, BDNF: brain-derived neurotropic factor, BLM: bleomycin, DSS: dextran sulfate sodium, FiO2: fraction of inspired oxygen , GSK-3β: glycogen synthase kinase 3 beta, HGF: hepatocyte growth factor, IC: intracranial, ICAM-1: intracellular adhesion molecule 1, IL: interleukin, IV: intravenous, MenSC: menstrual-derived stem cells, MI: myocardial infarction, MMP: matrix metalloproteinase, mtDNA: mitochondrial deoxyribonucleic acid, paO2: arterial pressure of oxygen, ROS: reactive oxygen species, SCC: squamous cell carcinoma , SCI-spinal cord injury, sO2%: oxygen saturation, TNF: tumour necrosis factor, TRAIL: tumour necrosis factor-related apoptosis-inducing ligand, VEGF: vascular endothelial growth factor.

Table 4: Clinical studies involving potential applications of menstrual derived stem cells in humans.

Disease	Human participant details and route of administration	Findings after MenSC treatment
Multiple sclerosis	4 patients suffering from MS injected intravenously or intrathecal [16]	• No immediate immune-reactivity or ectopic tissue formation at injection site • No abnormalities caused by MenSC administration was observed on physical examination, biochemical tests and chest X-ray • No objective neurological disease progression up to the date of publication
Duchene muscular dystrophy	Combination treatment of MenSC and CD34 umbilical cord blood [112]	• No adverse reaction • Increased number of muscle cells • Improved upper extremity function • Decrease of respiratory infections • Normal levels of dystrophin from a muscle biopsy
Congestive heart failure	• 60 patients suffering from non-ischaemic and ischaemic CHF. Delivery via retrograde coronary sinus [118] • 1 patient injected with MenSC and cord blood via IV [119]	• No serious adverse event • Improvement of ejection fraction • Reduction in pro-brain natriuretic peptide • Questionnaire score of the Minnesota living with heart failure was decreased • No abnormalities observed on physical examination and chest x-ray
H7N9	17 patients [126]	• Higher survival rate after treatment when compared to control group • No significant difference in the functions of FVC, FEV and forced expiratory flow at 50% vital capacity • Improvement in Hb levels and decrease in PT levels • Improvement on CCT

COVID-19	26 severe and critically ill patients injected with 3 infusions of MenSC from the same donor [125]	• Improvement of cough from day 1 • Expiratory dyspnoea improvement from day 1 • Higher survival rate when compared to control (92.31% survival for MenSC treatment and 66.67% survival for control population) • Treated group improved in their medical condition by 5.8 days shorter than control • 85% of patients treated with menstrual stem cells showed CCT improvement compared to the 50% from the control group • Amelioration of fibrosis • Improved SaO_2 and PaO_2
	1 patient [22]	• Reduced inflammatory cytokines • Clinical condition improvement • Improvement of consolidations that where present in the lung

Legend: CCT: chest computer tomography, CD34: cluster of differentiation 34, CHF: congestive heart failure, FEV: forced expiration volume, FVC: forced vital capacity, Hb: haemoglobin, IV: intravenous, MS: multiple sclerosis; PT: prothrombin time.

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Figure 1: PRISMA flow diagram study identification.

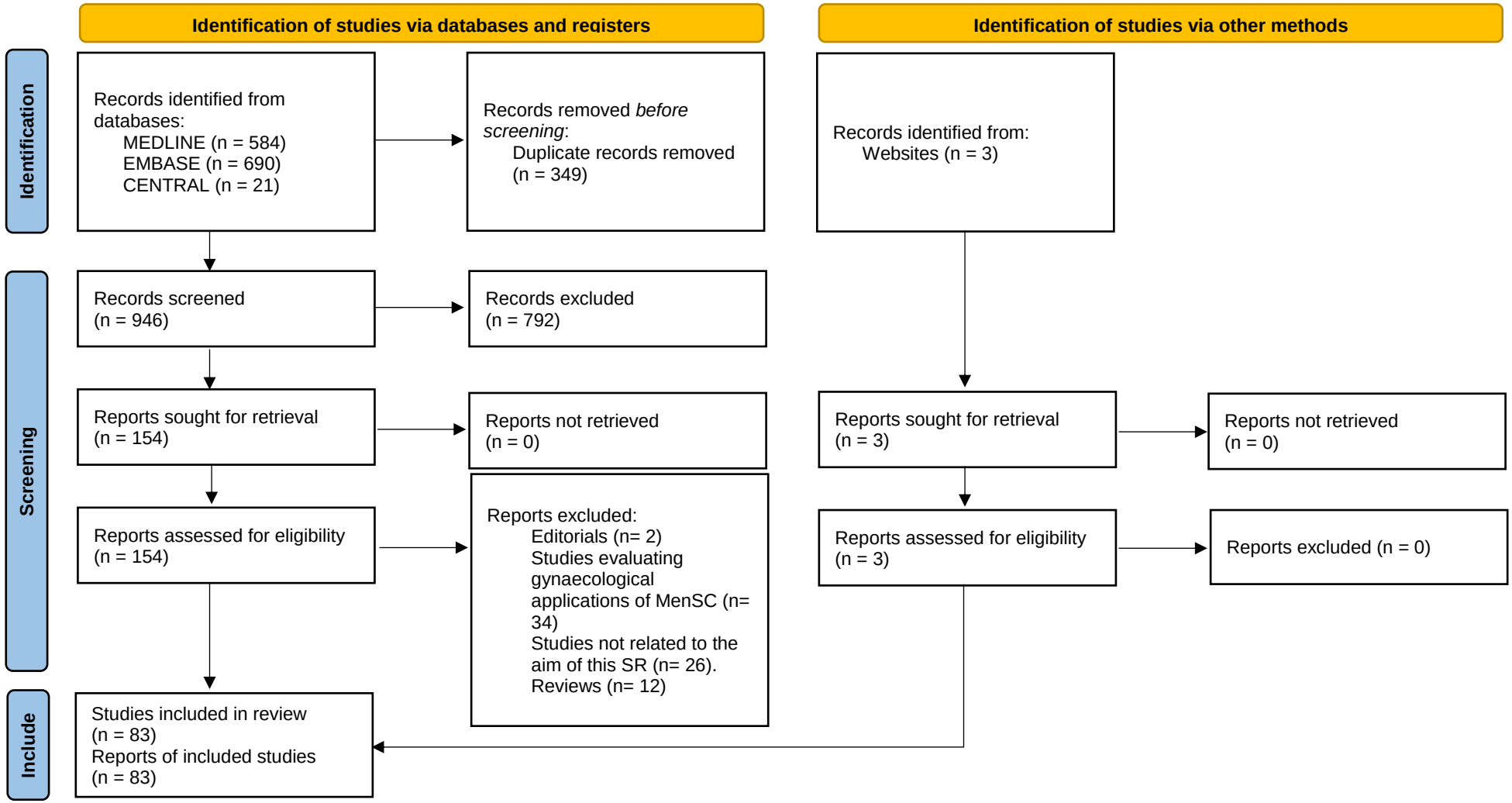

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Table 1: MEDLINE (Ovid) (from 2007 to August 2020, Week 4)

	Search	***Results***
1	Menstrual blood-derived stem cell*.mp.	37
2	Menstrual blood-derived stromal stem cell*.mp.	3
3	Menstrual blood-derived mesenchymal stem cell*.mp.	15
4	Menstrual blood-derived endometrial stem cell*.mp.	4
5	Menstrual blood-derived cell*.mp.	3
6	Menstrual blood-derived stromal cell*.mp.	4
7	Menstrual blood-derived progenitor cell*.mp.	0
8	Menstrual blood-derived regenerative cell*.mp.	0
9	Menstrual stem cell*.mp.	5
10	Menstrual blood stem cell*.mp.	23
11	Menstrual blood stromal stem cell*.mp.	3
12	Menstrual blood progenitor cell*.mp.	1
13	Menstrual-derived stem cell*.mp.	2
14	Endometrial stem cell*.mp.	139
15	Endometrial stromal stem cell*.mp.	7
16	Endometrial mesenchymal stem cell*.mp.	59
17	Endometrial progenitor cell*.mp.	8
18	Endometrial regenerative cell*.mp.	17
19	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18	286
20	exp Menstruation/	15,751
21	exp Endometrium/	32,210
22	20 or 21	46,533
23	exp Stem Cells/	216,632
24	exp Mesenchymal Stem Cells/	37,597
25	23 or 24	216,632
26	22 and 25	571

27	19 or 26	660
28	limit 27 to yr="2007 - 2020"	612
29	limit 28 to english language	584

Table 2: EMBASE (from 2007 to August 2020, week 4)

	Search	***Results***
#1	'menstrual blood-derived stem cell*'	68
#2	'menstrual blood-derived stromal stem cell*'	4
#3	'menstrual blood-derived mesenchymal stem cell*'	34
#4	'menstrual blood-derived endometrial stem cell*'	7
#5	'menstrual blood-derived cell*'	5
#6	'menstrual blood-derived stromal cell*'	8
#7	'menstrual blood-derived progenitor cell*'	0
#8	'menstrual blood-derived regenerative cell*'	0
#9	'menstrual stem cell*'	15
#10	'menstrual blood stem cell*'	59
#11	'menstrual blood stromal stem cell*'	10
#12	'menstrual blood progenitor cell*'	1
#13	'menstrual-derived stem cell*'	8
#14	'endometrial stem cell*'	320
#15	'endometrial stromal stem cell*'	15
#16	'endometrial mesenchymal stem cell*'	144
#17	'endometrial progenitor cell*'	12
#18	'endometrial regenerative cell*'	32
#19	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18	632
#20	'menstruation'/exp	23,693
#21	'endometrium'/exp	34,082
#22	#20 OR #21	56,053
#23	'stem cell'/exp	378,513
#24	'mesenchymal stem cell'/exp	60,648

#25	#23 OR #24	378,513
#26	#22 AND #25	808
#27	#19 OR #26	1,212
#28	#27 AND ([conference abstract]/lim OR [conference paper]/lim OR [conference review]/lim)	434
#29	#27 NOT #28	778
#30	#29 AND [english]/lim	728
#31	#30 AND [2007-2020]/py	690

Table 3: Cochrane Central Register of Controlled Trials (EBSCO) (from 2007 to August 2020, week 4)

	Search	***Results***
S1	Menstrual blood-derived stem cell*	4
S2	Menstrual blood-derived stromal stem cell*	1
S3	Menstrual blood-derived mesenchymal stem cell*	1
S4	Menstrual blood-derived endometrial stem cell*	0
S5	Menstrual blood-derived cell*	4
S6	Menstrual blood-derived stromal cell*	1
S7	Menstrual blood-derived progenitor cell*	0
S8	Menstrual blood-derived regenerative cell*	0
S9	Menstrual stem cell*	8
S10	Menstrual blood stem cell*	5
S11	Menstrual blood stromal stem cell*	2
S12	Menstrual blood progenitor cell*	0
S13	Menstrual-derived stem cell*	0
S14	Endometrial stem cell*	12
S15	Endometrial stromal stem cell*	0
S16	Endometrial mesenchymal stem cell*	7
S17	Endometrial progenitor cell*	5
S18	Endometrial regenerative cell*	0
S19	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18	21
S20	(MW menstrual) OR (MW menstruation)	1,489
S21	(MW endometrial) or (MW endometrium)	1,673
S22	S20 or S21	3,047
S23	MW stem cells	2,493
S24	MW mesenchymal stem cells	213
S25	S23 OR S24	2,493
S26	S22 AND S25	1

S27	S19 OR S26	22
S28	S27 **Limiters** - Published Date: 20070101-20201231	21