Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
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Mini-review:

Plasticity of human menstrual blood stem cells derived from the endometrium*

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Received Jan. 11, 2011; Revision accepted Apr. 1, 2011; Crosschecked Apr. 12, 2011

Abstract: Stem cells can be obtained from women's menstrual blood derived from the endometrium. The cells display stem cell markers such as Oct-4, SSEA-4, Nanog, and c-kit (CD117), and have the potent ability to differentiate into various cell types, including the heart, nerve, bone, cartilage, and fat. There has been no evidence of teratoma, ectopic formation, or any immune response after transplantation into an animal model. These cells quickly regenerate after menstruation and secrete many growth factors to display recurrent angiogenesis. The plasticity and safety of the acquired cells have been demonstrated in many studies. Menstrual blood-derived stem cells (MenSCs) provide an alternative source of adult stem cells for research and application in regenerative medicine. Here we summarize the multipotent properties and the plasticities of MenSCs and other endometrial stem cells from recent studies conducted both in vitro and in vivo.

Key words: Menstrual blood-derived stem cells (MenSCs), Endometrium, Multipotent, Plasticity
doi:10.1631/jzus.B1100015 **Document code:** A **CLC number:** Q813; R51

1 Introduction

Stem cells are defined as undifferentiated cells that are able to replicate themselves without differentiating (self renewal). Under some specific conditions, they can be induced to differentiate into various functional cell lineages, including adipocytes, chondrocytes, osteoblasts, smooth muscle cells, cardiomyocytes, neurons, and hepatocytes.

Depending on the stage of development, stem cells can be classified as either embryonic stem cells or adult stem cells (or somatic stem cells). Since the establishment of the first mice embryonic stem cell lines (Evans and Kaufman, 1981), researches on embryonic stem cells and the mesenchymal stem cells have shown great promises for cell-based therapeutics, such as wound healing (Fu *et al*., 2006; Lau *et al*., 2009; Luo *et al*., 2010), restoration of brain dopamine levels in a murine Parkinson's model (Wolff *et al*., 2010), acceleration of blood glucose levels (Piacibello *et al*., 1999; Arai *et al*., 2004), treatments of spinal cord injury (Nandoe Tewarie *et al*., 2006; Vaquero

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* Project supported by the National Science and Technology Major Project of China (No. 2008ZX10002-009), and the National Basic Research Program (973) of China (No. 2007CB513001)

and Zurita, 2009; Yamazaki *et al*., 2010) and a number of other diseases (Patel *et al*., 2005; Pitini *et al*., 2006; Vilquin and Rosset, 2006; Granero-Molto *et al*., 2008; Borlongan *et al*., 2010; Lee *et al*., 2010; Yang *et al*., 2010). However, embryonic stem cell studies face the hurdles of teratoma formation and the ethical controversies over the creation, usage, and destruction of human embryos. Being totipotent cells, embryonic stem cells can differentiate into many different types of cells and may cause a teratoma if injected directly into another body. One alternative is to explore the application of somatic stem cells and determine their full potential.

One of the methods for obtaining multipotent stromal stem cells is through extraction of bone marrow mesenchymal cells. This, however, poses a significant challenge in that the procedure is extremely invasive and would strictly limit the use of the cells in research. To circumvent this problem, a highly proliferative stem cell was identified in menstrual blood. It is a multipotent stromal stem cell from the endometrium maintaining the potential to differentiate (Meng *et al*., 2007). Here we review the advancement on endometrial stem cells (or menstrual blood-derived stem cells, MenSCs), which can exhibit stem/progenitor cells properties in vitro. Our animal studies show that the human endometrial stem cells can repair several types of damaged cells in vivo (unpublished data). Human stem cells harvested directly from endometrium were first described by Gargett (2004).

2 Generation and characteristics of MenSCs

Mesenchymal stem cells can be obtained from the uterus in a variety of ways including hysterectomy, diagnostic curettage, menstrual blood, and first-trimester decidua (Schüring *et al*., 2011). To date, the most convenient source of stem cells is isolated from menstrual blood. It is obvious that human endometrium shows a strong ability to regenerate, but the cellular sources that are responsible are still not understood (Aghajanova *et al*., 2010). Thus, it is important to study the nature of these cells in order to reveal their functions.

Inactive endometrium contains clonogenic epithelial and stromal cells that were demonstrated for the first time by Schwab *et al*. (2005). Endometrial stem cells, which were isolated from menstrual blood, also possess the adult stem cell-like characteristics of self-renewal, high proliferative potential in vitro, and the ability to differentiate towards diverse cell lineages in the induction media (Meng *et al*., 2007). Menstruation occurs typically about every 28 d in fertile women and some other female primates. During this period of time, the uterus experiences cyclical changes of endometrial thickening, vascular proliferation, glandular secretion, and endometrial growth, followed by the shedding of the functionalis of the endometrium. The menstrual cycle is regulated by hormone levels, primarily progesterone and estrogen in the proliferative and secretory phases (Toyoda *et al*., 2007). As hormone levels drop, endometrial support is suddenly lost, which results in vasoconstriction, necrosis of the endometrium, and finally, menstruation (Sherman and Korenman, 1975).

2.1 Definition of MenSCs

The uterine decidua is enriched in the menstrual blood, which then raises these questions: where do the adherent cells mainly originate? Are they homologous? Cui *et al*. (2007), who demonstrated skeletal muscle differentiation of MenSCs in the murine model cure for Duchenne muscular dystrophy, reported that menstrual blood in decidua contains a heterogeneous population of cells rather than dead stem cells (Prianishnikov, 1978). Endometrium consists of the epithelial layer and the underlying lamina propria. The lamina propria is structurally and functionally divided into the functionalis, which contains glands extending from the surface epithelium loosely held together by supportive stroma, and the lower basalis consisting of basal regions of the glands, dense stroma, and lymphoid aggregates (Gargett, 2007). The upper two-thirds of the functionalis of the endometrium are shed during menstruation. In a recent study, fibroblasts that originate from the endometrium were proven to have no regenerative capacity (Li *et al*., 2010). Mononuclear cells derived from bone marrow hematopoietic stem cells develop in the marrow cavity and remain in the blood for 2–3 d and subsequently migrate into surrounding tissues. Hence, menstrual blood may contain circulating bone marrow-derived mesenchymal stem cells (Taylor, 2004). Stem cells in endometrium, also considered

mononuclear cells, can be separated from blood cells with the modified selection media (Delo *et al*., 2006). In addition, phenotypic analysis of endometrial stem cells revealed none of the hematopoietic stem cell markers such as CD34 and CD45. The medium was changed every third day, and the cells could be eventually purified. The cells are referred to as MenSCs, stem cells derived from menstrual blood which share some of the same characteristics for adhesion and plasticity as other endometrial cells (Borlongan *et al*., 2010).

Recent studies have provided ample evidences for the existence of stem/progenitor cells present in human endometrium. Complete characterization of uterine stem/progenitor cells will improve our understanding of the support mechanisms of physiological regeneration in the female reproductive tract (Maruyama *et al*., 2010). Human uterine endometrial cells were once established as a feeder layer to maintain the undifferentiated state of human embryonic stem cells, and the high expressions of embryotrophic factors and extracellular matrices play a vital role in their growth (Lee *et al*., 2005). Although the current understanding of cyclic endometrium renewal is fragmentary, it is possible to construct a hypothesis of cyclic endometrial growth. What is the evidence for the putative stem cell existence in the endometrium? Human endometrium must contain a population of stem cells responsible for its remarkable regenerative ability as shown by Padykula (1991) in their surgical endometrectomy of mature rhesus monkeys. Thus, similar to what has been previously reported with bone marrow (Jiang *et al*., 2002), menstrual blood contains a population of cells that can be expanded in culture and is able to express the phenotype of multiple lineages.

2.2 Characteristics of MenSCs

Adherent cells have been isolated from menstrual blood collected from females during the heaviest flow of their cycle (Meng *et al*., 2007). These cells displayed stromal cell morphology in the serum-containing growth medium in a flask and doubled in number every 24 h. Chang's media for adhesion and proliferation were evidenced to be effective (Chang *et al*., 1982; Delo *et al*., 2006; Patel *et al*., 2008). Mesenchymal surface markers, including cluster of differentiation 9 (CD9), CD29, CD44, CD49f, CD90, CD105, CD117, CD166, major histocompatibility complex class I (MHC I), and multipotent markers octamer-binding transcription factor 4 (Oct-4; the main regulator of differentiation in pluripotent cell line), stage-specific embryonic antigen 4 (SSEA-4; the marker used to differentiate between human and mouse cells and used to characterize pluripotent stem cells), and stromal-derived factor-1 (STRO-1; used as identification, isolation, and functional testing of human bone marrow stromal progenitor cells) (Meng *et al*., 2007; Patel *et al*., 2008), were identified under fluorescent microscope or by flow cytometry.

Mesenchymal stem cells should also have their appropriate functional properties, not just the expressions of markers. Thus, a nuclear karyotype analysis and potential migration study should be performed. Stem cells derived from the menstrual blood can be largely expanded in vitro without any mutation or some other visible abnormality at the chromosomal level (Meng *et al*., 2007; Patel *et al*., 2008). They maintain greater than 50% of telomerase activity even at passage 12 compared with that in human embryonic stem cells (Patel *et al*., 2008), and also appear to mildly express the chemokine receptor CXCR4 and the respective receptor for stromal cell-derived factor-1 (SDF-1), which play a significant role in the mediation of mesenchymal stem cell migration (Meng *et al*., 2007; Patel *et al*., 2008).

There are reports investigating the feasibility of allogeneic transplantation into four cases of patients, using MenSCs (Zhong *et al*., 2009). No immune response or related side effects were found after a one-year follow-up. This demonstrates that the low immunogenicity of MenSCs is more or less able to suppress the immune reaction in vivo. One-way mixed lymphocyte culture has been prepared to detect the immunosuppressive properties of menstrual blood derivative (Borlongan *et al*., 2010). The low immunogenicity may be related to their immature immune system or the capacity of secreting immunosuppressive factors.

Overall, MenSCs are a unique cell population that can be safely isolated and provide an expandable source of stem cells from child-bearing aged women (Patel *et al*., 2008). Study of the characteristics of MenSCs would provide a new insight into the future treatment of various diseases.

3 Plasticity of stem cells

3.1 Definition of stem cell plasticity

With the study of stem cell biology progressing, researchers have found that some organs not only regenerate themselves, but also are capable of generating cells of other tissues under certain circumstances (Wagers and Weissman, 2004; Xiang *et al*., 2007). This ability is called cross-system development and differentiation: plasticity. To prove the plasticity of adult stem cells, two conditions must be satisfied: (1) the self-renewal and ability to differentiate into at least one functional cell type of specific tissue, and (2) the concomitant loss of original tissue-specific markers and function, and acquisition of markers and function of the new cell type (Gargett, 2007). Lakshmipathy and Verfaillie (2005) proposed three criteria for the definition of plasticity: (1) differentiation of a single cell into multiple cell lineages (Table 1), (2) functionalities of differentiated cells in vitro and in vivo, and (3) robustness and persistent engrafting of transplanted cells.

Plasticity of adult stem cells can be summarized mainly by the mechanisms of trans-differentiation, dedifferentiation, homogeneity, and cell-cell fusion (Bjornson *et al*., 1999; Tanaka, 2003; Wang *et al*., 2003). Previously thought to be associated with transcription factors and extracellular signal transduction, the stem cell plasticity is also subject to its regulation of the niches in which they reside. The niche provides support for the self-renewal and differentiation capabilities of the stem cells (Walker *et al*., 2009).

Pluripotency refers to a stem cell that has the potential to differentiate into any cell line of the three germ layers. Pluripotent adult stem cells are rare and generally small in number but can be found in a number of tissues including umbilical cord blood and menstrual blood.

3.2 Establishment of micro-environment for differentiation

Cells proliferate quickly by continuous subculture in vitro, but this limits the characteristics of cell differentiation. Cultured cells may lose the capacity of expressing phenotypes of the cells they rooted in and also their functional abilities (Stock *et al*., 2010). Thus, establishing the niches in vitro becomes the blue print of stem cell differentiation. The right simulation systems contain increased density of cells plated, intensive interactions of cell-cell and cell-matrix, and the addition of various differentiation factors in the culture medium. Numerous cytokines and growth factors have been shown to have a potent

Table 1 Pluripotent differentiation of embryonic stem cells and adult stem cells

Stem cell types	Cell types already differentiated from stem cells	References
ESCs	All three embryonic germ layers	Reubinoff *et al*., 2000
ASCs, HSCs	Blood cells, hepatocytes, cardiomyocytes, and epithelial cells, etc.	Ogawa, 1993; Lagasse *et al*., 2000; Krause *et al*., 2001; Orlic *et al*., 2001
BMMSCs	Adipocytes, osteoblasts, chondrocytes, cardiomyocytes, hepatocytes, neurons, and epithelial cells, etc.	Krause *et al*., 2001; Rebelatto *et al*., 2008
ADSCs	Adipocytes, osteoblasts, chondrocytes, cardiomyocytes, hepatocytes, and neurons, etc.	Gimble and Guilak, 2003; Strem *et al*., 2005; Rebelatto *et al*., 2008
PlaSCs	Chondroblasts, osteoblasts, adipocytes, myocytes, neuronal cells, and hepatocytes, etc.	Chien *et al*., 2006; Portmann-Lanz *et al*., 2006
UCBSCs	Adipocytes, osteoblasts, chondrocytes, and hepatocytes, etc.	Rebelatto *et al*., 2008; Moon *et al*., 2009
NSCs	Neurons, gliacytes, oligodendrocytes, myocytes, and hematopoietic cells, etc.	Kennea and Mehmet, 2002
SMSCs	Osteoblasts and adipocytes, etc.	Abdallah *et al*., 2004
AFSCs	Fibroblasts, adipocytes, and osteocytes, etc.	In′t Anker *et al*., 2003
MenSCs	Adipocytes, osteoblasts, chondrocytes, neurons, endotheliocytes, pulmonary epithelial cells, hepatocytes, islet cells, cardiac myocytes, and insulin-producing cells, etc.	Meng *et al*., 2007; Hida *et al*., 2008; Patel *et al*., 2008; Li *et al*., 2010

ESCs: embryonic stem cells; ASCs: adult stem cells; HSCs: hematopoietic stem cells; BMMSCs: bone marrow-derived mesenchymal stem cells; ADSCs: adipose tissue-derived stem cells; PlaSCs: placenta-derived stem cells; UCBSCs: umbilical cord-blood-derived stem cells; NSCs: neural stem cells; SMSCs: skeletal muscle stem cells; AFSCs: amniotic fluid-derived stem cells; MenSCs: menstrual blood-derived stem cells

effect on stem cell differentiation in vitro. However, these factors are difficult to determine because they are so complicated and numerous (Ikegami *et al*., 2010). Furthermore, the timing, dosage, combination, and the introduction of appropriate extracellular matrix (ECM) in vitro differentiation may largely affect the differentiation process (Banas *et al*., 2007). It may also be anticipated that any given differentiation condition in vitro will not fully reconstitute the environment (Stock *et al*., 2010). The differentiation potential of endometrial stem cells has been investigated by many groups in recent years (Table 1).

3.3 Differentiation into multiple cell lines

The multipotency of MenSCs has been demonstrated by directly differentiating them into chondrogenic, adipogenic, osteogenic, neurogenic, and cardiogenic cell lineages using the specific human mesenchymal stem cell differentiation bullet kit (Patel *et al*., 2008). The induction efficiencies have been evaluated. Tissue-specific markers of target cells were detected at the cellular and molecular levels. These studies laid the status of application of MenSCs in regenerative medicine. Similar work done by Meng *et al*. (2007)'s group has demonstrated that MenSCs are capable of differentiating in standard culture reagents into nine lineages: cardiomyocytic, respiratory epithelial, neurocytic, myocytic, endothelial, pancreatic, hepatic, adipocytic, and osteogenic. They found that these stem cells can produce matrix metalloproteinase-3 (MMP3), MMP10, granulocyte macrophage colony-stimulating factor (GM-CSF), angiopoietin-2, and platelet-derived growth factor (PDGF)-BB in quantities 10 to 10000 times higher than umbilical cord blood cells.

3.4 Myogenic differentiation for myocardial infarction, Duchenne muscular dystrophy, and limb ischemia

The pluripotency of human endometrial-derived stem cells has been demonstrated in vivo and in vitro. Optimization of the induction system is now an important focus for stem cell research. Ikegami *et al*. (2010) recently claimed that they have established a fetal bovine serum (FBS)-free cardiomyogenic transdifferentiation assay system in vitro. They confirmed that the induction efficiency is greatly improved and is surprisingly at a higher level compared to that in serum-containing medium (Hida *et al*., 2008). After a one-month proliferation, mesenchymal cells from nine different subjects' menstrual blood, co-cultured for 3 d with rat cardiocytes, began to beat naturally, exhibited cardiomyocyte-specific action potentials, and eventually transformed into a sheet of cardiocytes. The nurtured cells were transplanted into mice with myocardial infarction, which significantly improved their condition. The next step is to determine the key factors involved in converting mesenchymal cells in myocardial cells.

Toyoda *et al*. (2007) reported on myogenic differentiation of MenSCs and cell fusion with host muscle cells in treating muscular dystrophy to restore dystrophin.

In other studies, endothelial progenitor cells and bone marrow mesenchymal stem cells were used to treat limb ischemia (Kalka *et al*., 2000; Iwaguro *et al*., 2002). Vascular injection of cells into the sites of ischemia significantly improved limb ischemia with angiogenesis in the ischemic area. This has also been confirmed by Murphy *et al*. (2008) using MenSCs. The MenSCs in mice ischemic segments displayed the following characteristics: production of high level of growth factor and matrix metalloproteinase, inhibition of the inflammatory response, and proliferation and differentiation without losing any function. Although basic research indicates that the use of stem cells in improving the microcirculation is not very effective, it offers a new venue for those who may not respond to artery by-pass surgery.

3.5 Pancreatic differentiation for type I diabetes mellitus

In a recent well-designed study, endometrial mesenchymal stem cells (EMSCs) were induced to produce not only mesodermal and neuroectodermal lineage cells, but also pancreatic lineage cells in the serum-free modified pancreatic selection media. Microarray analysis showed that the expression levels of 716 genes changed between the primary and induced cells. Furthermore, a streptozotocin (STZ)-induced animal model of diabetes was used to demonstrate the ability of spheroid-like body (SB) EMSCs to engraft, differentiate in vivo, regulate blood glucose levels, and significantly prolong the survival of graft cells (Li *et al*., 2010).

In addition, routine endometrium biopsy can

also provide mesenchymal stem cell-like cells (Schüring *et al*., 2011). The putative endometrial cells possess the stem cell-like properties in the aforementioned studies. Osteogenic, adipogenic, and chondrogenic differentiations were performed by using the selection media.

3.6 Neurogenic differentiation for neurological disorders

Pathophysiologic alterations of Parkinson's disease are mainly due to the degeneration of dopamine neurons in substantia nigra, leading to reductions in dopamine (DA) levels in the striatum. While drug therapy and surgery do not fundamentally solve the problem, transplanting dopaminergic neurons into the brains of patients with Parkinson's disease yields more promising results. Endometrial stem cells, in Wolff *et al*. (2010)'s study, were used to generate dopaminergic neurons for the transplantation into Parkinson's disease model mice. These cells differentiated into nerve axons in vitro and expressed CD90, PDGF-Rβ, and CD146, and neural markers nestin and tyrosine hydroxylase, but no CD45 or CD31. This approach can exert neuroprotection and rectify the behavior disorders following transplantation in experimental Parkinson's disease, and the engraftment of DA neurons can survive and form functional connections in the brain. In the earlier research, endometrial cells were converted into cartilage cells in vivo (Wolff *et al*., 2007), which further proved the pluripotent potential of endometrial-derived mesenchymal cells. Those differentiated cells can express sulfate glycosaminoglycan and human articular cartilage collagen type II, the same as the normal cartilage cells (Wolff *et al*., 2007).

Stroke is one of the therapeutic indications of stem cells. $CD117^+$ cells selected from MenSCs were designed to target primary rat neurons injured by oxygen glucose deprivation (OGD) in Borlongan *et al*. (2010). After OGD and adenosine-triphosphate (ATP) activity assays for cell viability were performed, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for cell survival indicated neuroprotection of the MenSCs. Cells were then implanted directly into the striatum of a rat model for stroke induced by surgery. Behavioral tests, morphology analysis, and stem cell phenotypic marker analysis show that the treatment was successful 14 d post-transplantation. This provided evidence for the therapeutic potential of MenSCs and simultaneously confirmed that cell replacement is not the mode of action in this model.

Finally, robust expressions of specific markers and changes in cell morphologies of MenSCs occurred upon exposure to agents known to regulate stem cell differentiation, which supports the hypothesis that these cells may be an important source for scientific research. The differentiated cells with specific functions and the undifferentiated cells will migrate to damaged areas of the body, restoring some of the features of the site and also avoiding the side effects of traditional drug therapy.

4 Conclusions

Mesenchymal stem cells can be harvested from menstrual blood, uterine curettage, hysterectomy, or the routine endometrial biopsy. Stem cells obtained from menstrual blood avoid invasive procedures and the ethical controversy of embryonic stem cells. These cells display stem cell-like phenotypic markers and show great potential for self-renewal, plasticity, and undifferentiated proliferation for long periods of time in vitro. Menstrual cycle dependency of Oct-4, a marker involved in the self-renewal of undifferentiated embryonic stem cell, also exists in the endometrium and is independent of hormone-induced cyclical changes of the endometrium (Bentz *et al*., 2010). Compared with embryonic stem cells, the marker expressed in endometrium may not truly exhibit pluripotent features, including teratoma formation similar to the amniotic fluid stem cell (de Coppi *et al*., 2007; Borlongan *et al*., 2010). More importantly, the low immunogenicity and the absence of tumor formation have been observed in previous studies (unpublished data), suggesting that they are safe for scientific research.

A large number of studies have shown that MenSCs are a population of pluripotent cells. They are capable of differentiating into specific-tissue cells of three germ layers both in vitro and in vivo, and play an important role in improving the disease status of studied models. However, a significant portion of the current research on the plasticity of these cells has been based on the cell population without purification.

The population may involve many other cells that have not been identified. Reports to date have not indicated whether a single cell or cells with the same genotype possess these remarkable features of differentiation. Physical and chemical characteristics of stem cells are related to the regulation of their differentiation. Much still needs to be investigated to fully identify the superiority of MenSCs for basic research and clinical applications.

References

Abdallah, B., Jensen, C., Gutierrez, G., Leslie, R., Jensen, T., Kassem, M., 2004. Regulation of human skeletal stem cells differentiation by Dlk1/Pref-1. *J. Bone Miner. Res.*, **19**(5):841-852. [doi:10.1359/jbmr.040118]

Aghajanova, L., Horcajadas, J.A., Esteban, F.J., Giudice, L.C., 2010. The bone marrow-derived human mesenchymal stem cell: potential progenitor of the endometrial stromal fibroblast. *Biol. Reprod.*, **82**(6):1076-1087. [doi:10.1095/biolreprod.109.082867]

Arai, S., Minjares, C., Nagafuchi, S., Miyazaki, T., 2004. Improved experimental procedures for achieving efficient germ line transmission of nonobese diabetic (NOD)-derived embryonic stem cells. *Exp. Diabesity Res.*, **5**(3):219-226. [doi:10.1080/15438600490486877]

Banas, A., Yamamoto, Y., Teratani, T., Ochiya, T., 2007. Stem cell plasticity: learning from hepatogenic differentiation strategies. *Dev. Dyn.*, **236**(12):3228-3241. [doi:10.1002/dvdy.21330]

Bentz, E.K., Kenning, M., Schneeberger, C., Kolbus, A., Huber, J.C., Hefler, L.A., Tempfer, C.B., 2010. Oct-4 expression in follicular and luteal phase endometrium: a pilot study. *Reprod. Biol. Endocrin.*, **8**(1):38. [doi:10.1186/1477-7827-8-38]

Bjornson, C.R., Rietze, R.L., Reynolds, B.A., Magli, M.C., Vescovi, A.L., 1999. Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells in vivo. *Science*, **283**(5401):534-537. [doi:10.1126/science.283.5401.534]

Borlongan, C.V., Kaneko, Y., Maki, M., Yu, S.J., Ali, M., Allickson, J.G., Sanberg, C.D., Kuzmin-Nichols, N., Sanberg, P.R., 2010. Menstrual blood cells display stem cell-like phenotypic markers and exert neuroprotection following transplantation in experimental stroke. *Stem Cells Dev.*, **19**(4):439-452. [doi:10.1089/scd.2009.0340]

Chang, H., Jones, O., Masui, H., 1982. Human amniotic fluid cells grown in a hormone-supplemented medium: suitability for prenatal diagnosis. *PNAS*, **79**(15):4795. [doi:10.1073/pnas.79.15.4795]

Chien, C., Yen, B., Lee, F., Lai, T., Chen, Y., Chan, S., Huang, H., 2006. In vitro differentiation of human placenta-derived multipotent cells into hepatocyte-like cells. *Stem Cells*, **24**(7):1759-1768. [doi:10.1634/stemcells.2005-0521]

Cui, C.H., Uyama, T., Miyado, K., Terai, M., Kyo, S., Kiyono, T., Umezawa, A., 2007. Menstrual blood-derived cells confer human dystrophin expression in the murine model of Duchenne muscular dystrophy via cell fusion and myogenic transdifferentiation. *Mol. Biol. Cell*, **18**(5):1586-1594. [doi:10.1091/mbc.E06-09-0872]

de Coppi, P., Bartsch, G.Jr, Siddiqui, M.M., Xu, T., Santos, C.C., Perin, L., Mostoslavsky, G., Serre, A.C., Snyder, E.Y., Yoo, J.J., *et al.*, 2007. Isolation of amniotic stem cell lines with potential for therapy. *Nat. Biotechnol.*, **25**(1):100-106. [doi:10.1038/nbt1274]

Delo, D.M., de Coppi, P., Bartsch, G.Jr, Atala, A., 2006. Amniotic fluid and placental stem cells. *Methods Enzymol.*, **419**:426-438. [doi:10.1016/S0076-6879(06)19017-5]

Evans, M., Kaufman, M., 1981. Establishment in culture of pluripotential cells from mouse embryos. *Nature*, **292**(5819):154-156. [doi:10.1038/292154a0]

Fu, X., Fang, L., Li, X., Cheng, B., Sheng, Z., 2006. Enhanced wound-healing quality with bone marrow mesenchymal stem cells autografting after skin injury. *Wound Repair Regen.*, **14**(3):325-335. [doi:10.1111/j.1743-6109.2006.00128.x]

Gargett, C.E., 2004. Stem cells in gynaecology. *Aust. N. Z. J. Obstet. Gynaecol.*, **44**(5):380-386. [doi:10.1111/j.1479-828X.2004.00290.x]

Gargett, C.E., 2007. Uterine stem cells: what is the evidence? *Hum. Reprod. Update*, **13**(1):87-101. [doi:10.1093/humupd/dml045]

Gimble, J., Guilak, F., 2003. Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. *Cytotherapy*, **5**(5):362-369. [doi:10.1080/14653240310003026]

Granero-Molto, F., Weis, J.A., Longobardi, L., Spagnoli, A., 2008. Role of mesenchymal stem cells in regenerative medicine: application to bone and cartilage repair. *Expert Opin. Biol. Ther.*, **8**(3):255-268. [doi:10.1517/14712598.8.3.255]

Hida, N., Nishiyama, N., Miyoshi, S., Kira, S., Segawa, K., Uyama, T., Mori, T., Miyado, K., Ikegami, Y., Cui, C., *et al.*, 2008. Novel cardiac precursor-like cells from human menstrual blood-derived mesenchymal cells. *Stem Cells*, **26**(7):1695-1704. [doi:10.1634/stemcells.2007-0826]

Ikegami, Y., Miyoshi, S., Nishiyama, N., Hida, N., Okamoto, K., Miyado, K., Segawa, K., Ogawa, S., Umezawa, A., 2010. Serum-independent cardiomyogenic transdifferentiation in human endometrium-derived mesenchymal cells. *Artif. Organs*, **34**(4):280-288. [doi:10.1111/j.1525-1594.2009.00859.x]

In′t Anker, P.S., Scherjon, S.A., Kleijburg-van der Keur, C., Noort, W.A., Claas, F.H., Willemze, R., Fibbe, W.E., Kanhai, H.H., 2003. Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. *Blood*, **102**(4):1548-1549. [doi:10.1182/blood-2003-04-1291]

Iwaguro, H., Yamaguchi, J., Kalka, C., Murasawa, S., Masuda, H., Hayashi, S., Silver, M., Li, T., Isner, J., Asahara, T., 2002. Endothelial progenitor cell vascular endothelial growth factor gene transfer for vascular regeneration. *Circulation*, **105**(6):732-738. [doi:10.1161/hc0602.103673]

Jiang, Y., Jahagirdar, B.N., Reinhardt, R.L., Schwartz, R.E., Keene, C.D., Ortiz-Gonzalez, X.R., Reyes, M., Lenvik, T., Lund, T., Blackstad, M., *et al*., 2002. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*, **418**(6893):41-49. [doi:10.1038/nature00870]

Kalka, C., Masuda, H., Takahashi, T., Kalka-Moll, W., Silver, M., Kearney, M., Li, T., Isner, J., Asahara, T., 2000. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. *PNAS*, **97**(7):3422-3427. [doi:10.1073/pnas.070046397]

Kennea, N., Mehmet, H., 2002. Neural stem cells. *J. Pathol.*, **197**(4):536-550. [doi:10.1002/path.1189]

Krause, D., Theise, N., Collector, M., Henegariu, O., Hwang, S., Gardner, R., Neutzel, S., Sharkis, S., 2001. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell*, **105**(3):369-377. [doi:10.1016/S0092-8674(01)00328-2]

Lagasse, E., Connors, H., Al-Dhalimy, M., Reitsma, M., Dohse, M., Osborne, L., Wang, X., Finegold, M., Weissman, I.L., Grompe, M., 2000. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat. Med.*, **6**(11):1229-1234. [doi:10.1038/81326]

Lakshmipathy, U., Verfaillie, C., 2005. Stem cell plasticity. *Blood Rev.*, **19**(1):29-38. [doi:10.1016/j.blre.2004.03.001]

Lau, K., Paus, R., Tiede, S., Day, P., Bayat, A., 2009. Exploring the role of stem cells in cutaneous wound healing. *Exp. Dermatol.*, **18**(11):921-933. [doi:10.1111/j.1600-0625.2009.00942.x]

Lee, H.J., Lim, I.J., Lee, M.C., Kim, S.U., 2010. Human neural stem cells genetically modified to overexpress brain-derived neurotrophic factor promote functional recovery and neuroprotection in a mouse stroke model. *J. Neurosci. Res.*, **88**(15):3282-3294. [doi:10.1002/jnr.22474]

Lee, J.B., Lee, J.E., Park, J.H., Kim, S.J., Kim, M.K., Roh, S.I., Yoon, H.S., 2005. Establishment and maintenance of human embryonic stem cell lines on human feeder cells derived from uterine endometrium under serum-free condition. *Biol. Reprod.*, **72**(1):42-49. [doi:10.1095/biolreprod.104.033480]

Li, H., Chen, Y., Chen, S., Kao, C., Tseng, L., Lo, W., Chang, C., Yang, D., Ku, H., Twu, N., 2010. Induction of insulin-producing cells derived from endometrial mesenchymal stem-like cells. *J. Pharmacol. Exp. Ther.*, **335**(3):817-829. [doi:10.1124/jpet.110.169284]

Luo, G., Cheng, W., He, W., Wang, X., Tan, J., Fitzgerald, M., Li, X., Wu, J., 2010. Promotion of cutaneous wound healing by local application of mesenchymal stem cells derived from human umbilical cord blood. *Wound Repair Regen.*, **18**(5):506-513. [doi:10.1111/j.1524-475X.2010.00616.x]

Maruyama, T., Masuda, H., Ono, M., Kajitani, T., Yoshimura, Y., 2010. Human uterine stem/progenitor cells: their possible role in uterine physiology and pathology. *Reproduction*, **140**(1):11-22. [doi:10.1530/REP-09-0438]

Meng, X., Ichim, T.E., Zhong, J., Rogers, A., Yin, Z., Jackson, J., Wang, H., Ge, W., Bogin, V., Chan, K.W., *et al.*, 2007. Endometrial regenerative cells: a novel stem cell population. *J. Transl. Med.*, **5**(1):57. [doi:10.1186/1479-5876-5-57]

Moon, Y., Yoon, H., Lee, M., Jang, I., Lee, D., Lee, J., Lee, S., Lee, K., Kim, Y., Eom, Y., 2009. Multipotent progenitor cells derived from human umbilical cord blood can differentiate into hepatocyte-like cells in a liver injury rat model. *Tranplant. Proc.*, **41**(10):4357-4360. [doi:10.1016/j.transproceed.2009.08.053]

Murphy, M.P., Wang, H., Patel, A.N., Kambhampati, S., Angle, N., Chan, K., Marleau, A.M., Pyszniak, A., Carrier, E., Ichim, T.E., *et al.*, 2008. Allogeneic endometrial regenerative cells: an "off the shelf solution" for critical limb ischemia? *J. Transl. Med.*, **6**(1):45. [doi:10.1186/1479-5876-6-45]

Nandoe Tewarie, R.D., Hurtado, A., Levi, A.D., Grotenhuis, J.A., Oudega, M., 2006. Bone marrow stromal cells for repair of the spinal cord: towards clinical application. *Cell Transplant.*, **15**(7):563-577. [doi:10.3727/000000006783981602]

Ogawa, M., 1993. Differentiation and proliferation of hematopoietic stem cells. *Blood*, **81**(11):2844-2853.

Orlic, D., Kajstura, J., Chimenti, S., Jakoniuk, I., Anderson, S.M., Li, B., Pickel, J., McKay, R., Nadal-Ginard, B., Bodine, D.M., *et al.*, 2001. Bone marrow cells regenerate infarcted myocardium. *Nature*, **410**(6829):701-705. [doi:10.1038/35070587]

Padykula, H.A., 1991. Regeneration in the primate uterus: the role of stem cells. *Ann. N Y. Acad. Sci.*, **622**:47-56. [doi:10.1111/j.1749-6632.1991.tb37849.x]

Patel, A.N., Geffner, L., Vina, R.F., Saslavsky, J., Urschel, H.C.Jr, Kormos, R., Benetti, F., 2005. Surgical treatment for congestive heart failure with autologous adult stem cell transplantation: a prospective randomized study. *J. Thorac. Cardiovasc. Surg.*, **130**(6):1631-1638. [doi:10.1016/j.jtcvs.2005.07.056]

Patel, A.N., Park, E., Kuzman, M., Benetti, F., Silva, F.J., Allickson, J.G., 2008. Multipotent menstrual blood stromal stem cells: isolation, characterization, and differentiation. *Cell Transplant.*, **17(3)**:303-311. [doi:10.3727/096368908784153922]

Piacibello, W., Sanavio, F., Severino, A., Dane, A., Gammaitoni, L., Fagioli, F., Perissinotto, E., Cavalloni, G., Kollet, O., Lapidot, T., *et al.*, 1999. Engraftment in nonobese diabetic severe combined immunodeficient mice of human $CD34^+$ cord blood cells after ex vivo expansion: evidence for the amplification and self-renewal of repopulating stem cells. *Blood*, **93**(11):3736-3749.

Pitini, V., Altavilla, G., Arrigo, C., 2006. Surgical treatment for congestive heart failure with autologous adult stem cell transplantation. *J. Thorac. Cardiovasc. Surg*., **131**(5):1213-1214. [doi:10.1016/j.jtcvs.2005.12.048]

Portmann-Lanz, C., Schoeberlein, A., Huber, A., Sager, R., Malek, A., Holzgreve, W., Surbek, D., 2006. Placental mesenchymal stem cells as potential autologous graft for pre- and perinatal neuroregeneration. *Am. J. Obstet. Gynecol.*, **194**(3):664-673. [doi:10.1016/j.ajog.2006.01.101]

Prianishnikov, V.A., 1978. On the concept of stem cell and a

model of functional-morphological structure of the endometrium. *Contraception*, **18**(3):213-223. [doi:10.1016/S0010-7824(78)80015-8]

Rebelatto, C., Aguiar, A., Moretao, M., Senegaglia, A., Hansen, P., Barchiki, F., Oliveira, J., Martins, J., Kuligovski, C., Mansur, F., 2008. Dissimilar differentiation of mesenchymal stem cells from bone marrow, umbilical cord blood, and adipose tissue. *Exp. Biol. Med.*, **233**(7):901-913. [doi:10.3181/0712-RM-356]

Reubinoff, B., Pera, M., Fong, C., Trounson, A., Bongso, A., 2000. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. *Nat. Biotechnol.*, **18**(4): 399-404.

Schüring, A.N., Schulte, N., Kelsch, R., Röpke, A., Kiesel, L., Götte, M., 2011. Characterization of endometrial mesenchymal stem-like cells obtained by endometrial biopsy during routine diagnostics. *Fertil. Steril.*, **95**(1): 423-426. [doi:10.1016/j.fertnstert.2010.08.035]

Schwab, K.E., Chan, R.W., Gargett, C.E., 2005. Putative stem cell activity of human endometrial epithelial and stromal cells during the menstrual cycle. *Fertil. Steril.*, **84**(2): 1124-1130. [doi:10.1016/j.fertnstert.2005.02.056]

Sherman, B., Korenman, S., 1975. Hormonal characteristics of the human menstrual cycle throughout reproductive life. *J. Clin. Invest.*, **55**(4):699-706. [doi:10.1172/JCI107979]

Stock, P., Bruckner, S., Ebensing, S., Hempel, M., Dollinger, M.M., Christ, B., 2010. The generation of hepatocytes from mesenchymal stem cells and engraftment into murine liver. *Nat. Protoc.*, **5**(4):617-627. [doi:10.1038/nprot.2010.7]

Strem, B., Hicok, K., Zhu, M., Wulur, I., Alfonso, Z., Schreiber, R., Fraser, J., Hedrick, M., 2005. Multipotential differentiation of adipose tissue-derived stem cells. *Keio J. Med.*, **54**(3):132-141. [doi:10.2302/kjm.54.132]

Tanaka, E.M., 2003. Cell differentiation and cell fate during urodele tail and limb regeneration. *Curr. Opin. Genet. Dev.*, **13**(5):497-501. [doi:10.1016/j.gde.2003.08.003]

Taylor, H.S., 2004. Endometrial cells derived from donor stem cells in bone marrow transplant recipients. *JAMA*, **292**(1): 81-85. [doi:10.1001/jama.292.1.81]

Toyoda, M., Cui, C., Umezawa, A., 2007. Myogenic transdifferentiation of menstrual blood-derived cells. *Acta Myol.*, **26**(3):176-178.

Vaquero, J., Zurita, M., 2009. Bone marrow stromal cells for spinal cord repair: a challenge for contemporary neurobiology. *Histol. Histopathol.*, **24**(1):107-116.

Vilquin, J.T., Rosset, P., 2006. Mesenchymal stem cells in bone and cartilage repair: current status. *Regen. Med.*, **1**(4):589-604. [doi:10.2217/17460751.1.4.589]

Wagers, A.J., Weissman, I.L., 2004. Plasticity of adult stem cells. *Cell*, **116**(5):639-648. [doi:10.1016/S0092-8674(04)00208-9]

Walker, M., Patel, K., Stappenbeck, T., 2009. The stem cell niche. *J. Pathol.*, **217**(2):169-180. [doi:10.1002/path.2474]

Wang, X., Willenbring, H., Akkari, Y., Torimaru, Y., Foster, M., Al-Dhalimy, M., Lagasse, E., Finegold, M., Olson, S., Grompe, M., 2003. Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature*, **422**(6934): 897-901. [doi:10.1038/nature01531]

Wolff, E.F., Wolff, A.B., Hongling, D., Taylor, H.S., 2007. Demonstration of multipotent stem cells in the adult human endometrium by in vitro chondrogenesis. *Reprod. Sci.*, **14**(6):524-533. [doi:10.1177/1933719107306896]

Wolff, E.F., Gao, X.B., Yao, K.V., Andrews, Z.B., Du, H., Elsworth, J.D., Taylor, H.S., 2010. Endometrial stem cell transplantation restores dopamine production in a Parkinson's disease model. *J. Cell. Mol. Med.*, in press. [doi:10.1111/j.1582-4934.2010.01068.x]

Xiang, Y., Zheng, Q., Jia, B.B., Huang, G.P., Xu, Y.L., Wang, J.F., Pan, Z.J., 2007. Ex vivo expansion and pluripotential differentiation of cryopreserved human bone marrow mesenchymal stem cells. *J. Zhejiang Univ.-Sci. B*, **8**(2): 136-146. [doi:10.1631/jzus.2007.B0136]

Yamazaki, Y., Kanno, H., Maeda, K., Yoshida, T., Kobayashi, N., Kubo, A., Yamaguchi, Y., Saito, T., 2010. Engrafted VHL peptide-delivered bone marrow stromal cells promote spinal cord repair in rats. *Neuroreport*, **21**(4): 287-292. [doi:10.1097/WNR.0b013e328336ee9a]

Yang, M., Wei, X., Li, J., Heinel, L.A., Rosenwasser, R., Iacovitti, L., 2010. Changes in host blood factors and brain glia accompanying the functional recovery after systemic administration of bone marrow stem cells in ischemic stroke rats. *Cell Transplant.*, **19**(9):1073-1084. [doi:10.3727/096368910X503415]

Zhong, Z.H., Patel, A.N., Ichim, T.E., Riordan, N.H., Wang, H., Min, W.P., Woods, E.J., Reid, M., Mansilla, E., Marin, G.H., *et al.*, 2009. Feasibility investigation of allogeneic endometrial regenerative cells. *J. Transl. Med.*, **7**(1):15. [doi:10.1186/1479-5876-7-15]