Identification and Characterization of Human Endometrial Mesenchymal Stem/Stromal Cells and Their Potential for Cellular Therapy

SAEEDEH DARZI,a,b JEROME A. WERKMEISTER,b,c JAMES A. DEANE,a,b CAROLINE E. GARGETT*a,b

aThe Ritchie Centre, Hudson Institute of Medical Research, Clayton, Victoria, Australia; bDepartment of Obstetrics and Gynaecology, Monash University, Clayton, Victoria, Australia; cCommonwealth Scientific and Industrial Research Organisation, Clayton, Victoria, Australia

SUMMARY

Human endometrium is a highly regenerative tissue, undergoing more than 400 cycles of proliferation, differentiation, and shedding during a woman’s reproductive life. Adult stem cells, including mesenchymal stem/stromal cells (MSCs), are likely responsible for the immense cellular turnover in human endometrium. The unique properties of MSCs, including high proliferative ability, self-renewal, differentiation to mesodermal lineages, secretion of angiogenic factors, and many other growth-promoting factors make them useful candidates for cellular therapy and tissue engineering. In this review, we summarize the identification and characterization of newly discovered MSCs from the human endometrium: their properties, the surface markers used for their prospective isolation, their perivascular location in the endometrium, and their potential application in cellular therapies.

SIGNIFICANCE

The endometrium, or the lining of uterus, has recently been identified as a new and accessible source of mesenchymal stem cells, which can be obtained without anesthesia. Endometrial mesenchymal stem cells have comparable properties to bone marrow and adipose tissue mesenchymal stem cells. Endometrial mesenchymal stem cells are purified with known and novel perivascular surface markers and are currently under investigation for their potential use in cellular therapy for several clinical conditions with significant burden of disease.

INTRODUCTION

Mesenchymal stem/stromal cells (MSCs) are attractive candidates for cellular therapies. Initial enthusiasm centered on the capacity of MSCs to differentiate into mesodermal lineages and generate skeletal tissues [1]. It is now recognized that MSCs act in a paracrine manner, secreting multiple factors that stimulate angiogenesis, reduce fibrosis, and promote endogenous cell proliferation to effect tissue repair [2]. The immunomodulatory and anti-inflammatory properties of MSCs are also appealing [3, 4] and have been exploited for the treatment of graft-versus-host disease [4, 5] and to reduce graft failure in haploidentical transplants [3]; however, MSCs appear less effective in humans than in rodent preclinical studies [6].

The ability of systemically administered MSCs to home to injured tissues to exert their paracrine actions is another attractive feature [7]. By exerting immunomodulatory effects, MSCs have been used for bone and cartilage regeneration in osteoporotic fracture and arthritis, and also for repair of cardiac tissue after myocardial infarction [3], seemingly acting in a paracrine manner rather than directly differentiating into specialized cells to regenerate new tissue [8]. Despite these promising findings on the utility of MSCs as a cell-based therapy, more scientific studies are required to better characterize the MSC populations used (Table 1) [9].

Limitations on the clinical use of MSCs include their rarity in tissues necessitating in vitro expansion [10], donor age affecting their proliferative capacity [11], and invasive methods required for harvesting bone-marrow aspirates. Apart from bone marrow, MSCs for therapeutic use are commonly sourced from adipose and placental tissues using a variety of markers (Table 1).

Adipose tissue has recently emerged as an attractive source of MSCs for cell-based therapies, readily available in modern society and frequently harvested by relatively invasive liposuction procedures. Adipose-derived stem cells (ASCs) (Table 1) are relatively abundant in the stromal vascular fraction of fat tissue and are often used without culturing [12].

Human placenta also offers an easily accessible source MSC as it is medical waste tissue. Maternal MSCs are generally derived from the placental tissue [13], whereas fetal MSCs are obtained from the umbilical cord, amniotic membrane, and amniotic fluid.

Another source of MSCs is the endometrium, a highly remodeling tissue. Human endometrium is the mucosal lining of the uterus, which undergoes more than 400 cycles of regeneration, differentiation, and shedding during a woman’s reproductive life [14]. Each...
month, 5–10 mm of new endometrial mucosa grows during the first 4–10 days of the menstrual cycle from the residual basalis layer (0.5–1 mm thick) to generate a new functionalis layer into which the embryo subsequently implants (Fig. 1A). Within 48 hours of endometrial shedding, regeneration begins with rapid repair/re-epithelialization of the endometrial surface to cover the exposed basalis surface [15]. Fragments of shedding endometrial tissue contribute to this repair and gene profiling has demonstrated that the lysed stroma is enriched in genes involved in extracellular matrix biosynthesis and degradation [16]. As circulating estrogen levels rise during the proliferative stage of the menstrual cycle, epithelial, stromal, and vascular cells rapidly proliferate to generate the glands and supportive stroma of the rapidly growing functionalis layer. Following involution, the secretory phase of the cycle commences under the influence of progesterone to block cell division and promote terminal differentiation of the glandular epithelium. Stromal differentiation into decidual cells occurs around blood vessels and beneath the luminal epithelium into which an embryo implants. In the absence of an implanting embryo, the functionalis regresses and is shed as menstruation proceeds; then, a new cycle commences [19]. During the last 10 years, an MSC subpopulation has been identified and characterized in human endometrium and in menstrual blood. Endometrial mesenchymal stem/stromal cells (eMSCs) are easily isolated from endometrial biopsy tissue, which is obtained noninvasively without anesthesia in an office-based procedure. eMSCs are the focus of this perspective.

## Table 1. Comparison of phenotypic markers of endometrial, bone marrow, and adipose tissue MSCs isolated as CFU-F, by plastic adherence or by SUSD2 or CD34 cell sorting

| MSC type | CD29 | CD31 | CD34 | CD44 | CD45 | CD73 | CD90 | CD105 | CD140b | CD146 | STRO1 | HLA-DR | References |
|----------|------|------|------|------|------|------|------|-------|---------|-------|-------|--------|------------|
| Clonogenic eMSCs | >95 | — | 85 | 0 | 85 | 92 | 16 | 68.5 | 19 | 0 | — | [19] |
| BM plastic-adherent MSCs | >95 | — | 0 | — | 0 | ≥ 90 | ≥ 90 | ≥ 90 | >95 | 15-20 | 3.8 | 0 | [33, 45, 46] |
| Adipose tissue plastic-adherent MSCs | >95 | — | 64 | 0 | 25 | 55 | 5 | — | 21 | 0 | 1 | — | [47, 48] |
| CD146<sup>+</sup> eMSCs cultured in DMEM/EGF/FGF2 SFM | 95.1 | — | 0 | 86 | 0.8 | 79 | — | 92 | 69 | 37 | — | — | [43] |
| SUSD2<sup>+</sup> eMSCs, freshly isolated | 11.6 | 5.3 | — | 77 | 4.7 | 99 | 71 | 99 | 85 | 28 | 60 | — | [26] |
| SUSD2<sup>+</sup> eMSCs cultured in serum medium | 93 | 2.3 | 0.5 | 93 | — | 99.9 | 98 | 99.5 | 50 | 1-2 | 0.9 | 0 | [49], unpublished |
| SUSD2<sup>+</sup> bone marrow MSCs, freshly isolated | — | 26 | 5 | 70 | 90 | — | 0 | 20 | — | 4 | — | 22 | [50] |
| SUSD2<sup>+</sup> bone marrow MSCs cultured in serum medium | — | 5.33 | 1.4 | 83 | 1.6 | — | 81 | 64 | — | 95 | — | 1.7 | [50] |
| CD34<sup>+</sup> ASCs, freshly isolated | — | 38 | 78 | 81 | 57 | — | 39 | 28 | — | 41 | — | 10 | [50] |
| CD34<sup>+</sup> ASCs cultured in serum medium | — | 6.7 | 17 | 98 | 5.8 | — | 56 | 79 | — | 13 | — | 4.9 | [50] |

SUSD2 can be used for isolating endometrial and bone marrow MSCs but does not isolate clonogenic ASCs; however, CD34 does. Abbreviations: —, no data; ASC, adipose-derived stem cell; BM, bone marrow; CFU-F, colony-forming unit fibroblast; DMEM, Dulbecco’s modified Eagle’s medium; EGF, epidermal growth factor; eMSC, endometrial mesenchymal stem cell; FGF2, fibroblast growth factor 2; HLA-DR, human leukocyte antigen DR; MSC, mesenchymal stem/stromal cell; SFM, serum-free medium.

### Endometrial Mesenchymal Stem Cells

Human eMSCs were first identified in cell cloning studies from single cells dissociated directly from full thickness endometrium obtained from hysterectomy tissue (Fig. 1B). A small proportion of the stromal colonies were large (1.3%), comprising small, densely packed cells likely originating from stromal stem/progenitor cells or colony-forming unit fibroblast (CFU-F) [17, 18]. Single, large endometrial CFU-Fs showed high proliferative capability undergoing 30 population doublings before reaching senescence, generating $6.5 \times 10^{11}$ cells [19]. Single endometrial CFU-F serially propagated at very low cloning densities (5–10 cells/cm²) up to five times, demonstrating self-renewal in vitro [19]. Single endometrial CFU-F also differentiated into the classic mesodermal lineages (adipocyte, osteocyte, myocyte, and chondrocyte) and expressed the typical pattern of MSC surface markers, CD29, CD44, CD73, and CD90 and lacked CD31, CD34, and CD45 (Table 1) [20]. A greater percentage of CFU-F was observed in the proliferative stage of the menstrual cycle when the endometrium is rapidly growing under the influence of rising circulating estrogen levels. CFU-Fs were also detected in inactive, noncycling endometrium from women on oral contraceptives and from postmenopausal women, suggesting that stromal CFU-F may have a role in regenerating atrophic endometrial stroma when circulating estrogen levels are restored. Further evidence for eMSCs comes from label-retaining cells (LRCs) in mouse endometrium, which are perivascular and participate in endometrial regeneration [21].

### Prospective Isolation of Human eMSCs Using Specific Markers

Colocalization of CD140b and CD146 (pericyte markers) first identified eMSCs and their pericycle location in human endometrium [22]. These markers were also used to identify perivascular MSCs in many fetal and adult tissues [23]. Almost all the endometrial CFU-Fs are found in the CD140b<sup>+</sup>CD146<sup>+</sup>
Figure 1. Schematic showing procedures for eMSC isolation from human endometrium and menstrual blood. (A): eMSC location in human endometrium. eMSCs are perivascular cells in the germinatal basalis and in the menstruated functionalis layers. (B): Human eMSCs are isolated from hysterectomy and endometrial biopsy (office procedure) tissue by enzymes and mechanical dissociation; stromal cells are then sequentially negatively selected from endometrial epithelial cells using EpCAM magnetic beads and leukocytes removed using CD45 magnetic beads, followed by either FACS for CD140b−CD146+ as shown in the FACS plot or magnetic bead sorting for SUSD2+. Ovine eMSCs are sorted directly from dissociated endometrium collected from hysterectomy tissue using FACS for CD271−CD49f+ cells (CD49f removes contaminating epithelial cells) as shown in the FACS plot. The properties of the MSC isolated by each marker/marker combination are also indicated. Confocal images show CD140b−CD146+ pericytes (yellow, white arrows) and perivascular SUSD2+ (red, white arrows) cells in human endometrium. Scale bar = 50 μm. Adventitial perivascular CD271+ (red, white arrowheads) ovine eMSCs located adjacent to α-smooth muscle actin+ vascular smooth muscle cells (green) of an arteriole (a) in ovine endometrium (yellow arrowheads are nonvessel associated CD271+ cells). Scale bar = 20 μm. Endometrial stromal cells are directly cultured from menstrual blood as plastic-adherent cells in a similar manner to bone marrow. FACS plots and images reprinted from KE Schwab, CE Gargett, Co-expression of two perivascular cell markers isolates mesenchymal stem-like cells from human endometrium. Hum Reprod 2007;22:2903–2911, by permission from Oxford University Press (CD140b, CD146) [22], IngentaConnect (SUSD2) [26], and PLoS One (CD271) [32]. Abbreviations: eMSC, endometrial mesenchymal stem cell; EpCAM, epithelial cell adhesion molecule; FACS, fluorescence-activated cell sorting; FITC, fluorescein isothiocyanate; g, gland; MSC, mesenchymal stem/stromal cell; PDGF-Rβ, platelet-derived growth factor receptor β; PE, phycoerythrin; s, stroma.
fraction, with an 8-fold enrichment over unsorted stromal cells and 17-fold over CD140b\(^-\)CD146\(^-\) cells [22]. The CD140b\(^+\)CD146\(^+\) cells, constituting 1.5% of endometrial stromal cells, fulfilled the classic MSC criteria [24, 25].

Given the damaging effect of flow cytometry sorting on eMSC clonogenicity [22], a single marker was sought to enable selection with magnetic beads. Screening with antibodies marking the perivascular region identified the W5C5 antibody as a novel single marker for isolating multipotent, self-renewing eMSCs that reconstitute stromal and vascular tissue in vivo [26]. W5C5\(^+\) cells constitute 4.2% of the stromal population, almost 3 times more than the CD140b\(^-\)CD146\(^-\) population. W5C5 cells showed a 6-fold increase in CFU-F activity over unsorted endometrial stromal cells and an 18-fold increase over the W5C5\(^-\) cells, indicating that most clonogenic cells were in the W5C5\(^+\) population. The epitope recognized by the W5C5 antibody is Sushi-domain-containing-2 (SUSD2) [27].

SUSD2\(^+\) eMSCs were also identified in estrogen-regenerated and atrophic postmenopausal endometrium as clonogenic, multipotent, and serially clonogenic cells, although their MSC functional activity including clonogenicity, self-renewal, and differentiation ability was lower than premenopausal eMSCs [28]. An RNA sequencing study comparing cultured human endometrial SUSD2\(^+\) and SUSD2\(^-\) cells identified 12 marker genes reflecting the perivascular location of SUSD2\(^+\) cells [29]. A single marker of perivascular eMSCs enables their prospective isolation using magnetic bead sorting, an important consideration for their use in cellular therapies.

CD271, the low affinity nerve growth factor receptor, has been used to isolate bone marrow MSCs [30]. CD271\(^+\) MSCs are clonogenic, express the classic MSC surface markers, and are multipotent [30]. The CD271 antibody cross-reacts with clonogenic bone marrow MSCs in several large animals often used in preclinical studies: ovine, monkey, goat, canine, and porcine species [31]. CD271 isolates MSCs from ovine endometrium when used in combination with CD49f to exclude endometrial epithelial cells (Fig. 1B). Ovine CD271\(^+\)CD49f\(^-\) endometrial stromal cells were more clonogenic, underwent more rounds of serial cloning, and showed greater mesodermal differentiation than CD271\(^-\)CD49f\(^-\) stromal fibroblasts [32].

**EMSC In Vivo Identity**

eMSC activity is likely regulated by the niche microenvironment. A perivascular niche for eMSCs was first identified in 40% of stromal LRCs found near the endometrial-myometrial junction, many of which expressed \(\alpha\)-smooth muscle actin (\(\alpha\)-SMA), indicating their perivascular identity [20]. In human endometrium, both CD140b\(^-\)CD146\(^-\) and SUSD2\(^-\) cells are perivascular (Fig. 2) in both the functionalis and basalis layers (Fig. 1A). By confocal microscopy, CD140b\(^+\)CD146\(^+\) coexpressing eMSCs are pericytes (Figs. 1B, 2) [22]. The precise perivascular location of SUSD2\(^+\) cells is less clear. These are more abundant than CD140b\(^-\)CD146\(^-\) cells and possibly both pericytes and adventitial cells around larger vessels [26]. An adventitial, but not pericyte perivascular, location was identified for ovine CD271\(^+\) eMSCs in arterioles and larger venules (Figs. 1B, 2) [32]. Ovine CD271\(^+\) eMSCs are not in close apposition with vWF\(^+\) endothelial cells and did not express \(\alpha\)-SMA but were adjacent to \(\alpha\)-SMA\(^-\) vascular smooth muscle cells in the vessel media, similar to bone marrow MSCs and adipose-derived MSCs [23]. Whether SUSD2\(^+\) cells are located in both pericyte and adventitial perivascular regions remains to be determined.

**Menstrual Blood MSCs**

The endometrial functionalis layer is shed during menstruation. Given the location of CD140b\(^-\)CD146\(^-\) and SUSD2\(^+\) eMSCs in both the functionalis and basalis layers, it is not surprising that eMSCs are found in menstrual blood (Fig. 1B). Viable stromal cells with classic properties of MSCs have been cultured from menstrual blood. Menstrual blood stromal cells are plastic-adherent, multipotent, express the classic pattern of MSC surface markers, and retain a stable karyotype in culture [33].

Menstrual blood represents a source of eMSCs easily collected from waste tissue and cultured similarly to bone marrow aspirates; however, without selective marker enrichment as is achieved for endometrial biopsy-derived eMSCs, they may not be as consistent or as efficacious.

**Potential Clinical Application of eMSCs**

Cellular therapy is an emerging field in clinical medicine. Many adult stem cells or pluripotent cell derivatives are in clinical trials for treatment of chronic and degenerative disease, the most common being bone marrow MSCs followed by ASCs. Tissue engineering involves the generation of a tissue substitute comprising a scaffold seeded with stem/progenitor cells for restoring or maintaining normal tissue/organ function. Scaffolds provide a three-dimensional environment supporting MSC adhesion and growth, facilitating delivery of cells to target sites to promote the growth of new tissue. eMSCs could be an ideal candidate for seeding biomaterials because they are easily acquired in an office-based biopsy without anesthesia.
Pelvic organ prolapse (POP) is the herniation of the pelvic organs into the vagina causing urinary and fecal incontinence, and voiding and sexual dysfunction. POP affects 25% of all women and up to 50% of parous postmenopausal women. Nineteen percent of women have a lifetime risk of undergoing an operation for POP [34, 35]. Due to high failure rates of native tissue surgery, synthetic meshes became popular for POP treatment, particularly monofilament, nondegradable polypropylene (PP) meshes with large pore sizes. Significant adverse events resulting from the use of mesh for POP surgery, including exposure, contraction, and pain led the U.S. Food and Drug Administration to post several warnings on the use of vaginal mesh [34, 36]. To address these problems, we developed and evaluated a new polyamide/gelatin composite mesh (PA+G) with improved mechanical and tissue integration properties, compared with commercial PP meshes in a rat abdominal hernia repair model [37]. These PA+G meshes seeded with eMSCs, and implanted into a rat subcutaneous fascial defect model, promoted more neovascularization resulting in a higher density of α-SMA+ vessel profiles in the neotissue surrounding mesh filaments compared with unseeded PA+G mesh. There was an initial early leukocyte response mainly involving M1 inflammatory macrophages for both eMSC-seeded and unseeded meshes, which switched to a M2 wound healing phenotype only in eMSC/PA+G implanted tissues. In the long term (90 days), the macrophages at the mesh filament-tissue interface were markedly reduced in the eMSC/PA+G mesh compared with PA+G alone. Crimped, more organized collagen fibers were deposited around cell-seeded mesh suggesting that eMSCs might promote physiological collagen production, which led to improved biomechanical properties with a more compliant, less stiff mesh/tissue complex than PA+G mesh alone [38, 39]. Because the eMSCs persisted for only 2 weeks, new tissue formation was likely due to the paracrine effects of eMSCs. In vitro, PA+G mesh seeded with eMSCs differentiated into smooth muscle-myosin heavy chain-expressing smooth muscle cells and collagen-producing fibroblasts, with concomitant downregulation of SUSD2 expression [40]. Fibroblasts and smooth muscle cells are the desired cell types for regenerating the human vaginal wall and synthesizing and organizing the extracellular matrix. Based on this promising result in a xenogeneic, small animal pre-clinical model of POP repair surgery, a large animal ovine model is being developed to examine the efficacy and mechanism of action of PA+G mesh seeded with autologous ovine eMSCs [32] on vaginal repair of parous ewes with demonstrated vaginal wall weakness. Although there are many sources of MSCs for cell-based therapies, few studies have been conducted for pelvic organ repair and there are no clinical trials for POP. Evidence suggests that these cells, and in particular eMSCs, may offer an improved therapeutic option for POP in the near future.

Menstrual blood MSCs have been used to repair the heart using a tissue engineering approach in a myocardial infarct nude rat model [41]. Cultured enhanced green fluorescent protein-labeled menstrual blood MSCs were harvested as a sheet from temperature-sensitive culture plates and grafted onto the infarcted area. The MSCs differentiated into striated, troponin-I-expressing cardiac muscle cells. In comparison with bone marrow MSCs, the menstrual blood MSCs reduced the infarcted area more and showed greater improvement in echocardiographic parameters of myocardial function, indicating the potential promise of menstrual blood-derived MSCs for clinical translation.

The highly regenerative endometrium provides a new, readily available source of MSCs with capabilities similar to those of bone marrow MSCs. Endometrial tissue is easily harvested from women with minimal pain and morbidity [42]. Specific perivascular markers of eMSCs have been identified and methods developed to prospectively isolate them from endometrial cell suspensions and to expand them under Good Manufacturing Practice guidelines [43]. These developments and the increasing recognition of the uterus as a source of MSCs will ensure their use in autologous and allogeneic cellular therapy, as well as homologous tissue engineering applications for regenerating endometrium in thin endometrial infertility disorders and endometrial scarring (Asherman’s syndrome) [44].

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The authors indicated no potential conflicts of interest.

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