Endometrial Stem Cells and Their Applications in Intrauterine Adhesion

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Abstract

Intrauterine adhesion (IUA), resulting from pregnancy or nonpregnant uterine trauma, is one of the major causes of abnormal menstruation, infertility, or repeated pregnancy loss. Although a few methods, including hysteroscopy and hormone therapy, are routinely used for its diagnosis and treatment, they cannot restore tissue regeneration. Stem cells, which have self-renewal and tissue regeneration abilities, have been proposed as a promising therapy for patients with severe IUAs. In this review, we summarize the origin and features of endometrium-associated stem cells and their applications in the treatment of IUAs based on animal models and human clinical trials. We expect that this information will help to elucidate the underlying mechanism for tissue regeneration and to improve the design of stem cell–based therapies for IUAs.

Introduction

The human endometrium undergoes more than 400 cycles of menstruation during a woman's reproductive lifetime[1]. It consists of the functional layer, which accounts for two-thirds of the tissue on the surface, and the basal layer, which is located underneath. During the menstrual cycle, with the change in hormone levels (mainly estrogen and progesterone)[2], the functional layer is shedding periodically[3], leaving the remaining basal layer, approximately 1 to 2 mm, adjacent to the myometrium. After menstruation, endometrial tissue gradually returns to normal thickness as the levels of estrogen and progesterone increase. The increase in endometrial thickness is crucial to embryo implantation and growth[4] (Fig. 1).

Intrauterine adhesions (IUAs), also called Asherman syndrome if severe uterine adhesion occurs, are mainly due to abortion or postpartum bleeding curettage[5,6]. This leads to partial or complete occlusion of the uterine cavity[7]. IUAs can cause abnormal menstruation, hypomenorrhea and amenorrhea, infertility, or repeated pregnancy loss, depending on the severity[8–10]. At present, the primary goal of IUAs treatment is to reconstruct the anatomical structure and restore uterine function[11]. For example, balloon stents and sodium hyaluronate can effectively prevent the recurrence of IUA[12,13]. Hysteroscopy is an effective method for diagnosis and treatment as well[14,15]. However, hysteroscopy also has many side effects, including bleeding, shock, and death[16].

Although current IUA therapies have been proven to have clinical effectiveness, they contribute little or no to pregnancy outcomes. Studies have shown that one of the significant molecular causes of IUAs is the decrease in stem cells in the basal layer of the endometrium[17,18]. Stem cells capable of self-renewal and differentiation are the foundation for tissue homeostasis, repair, and regeneration. A recent study showed that endometrial stem cells extracted from IUAs patients have low angiogenic activity, clone formation, and proliferation compared with those from healthy females[19]. Stem cell therapy, which promotes the repair mechanism of the injured tissue by stem cells and their differentiated derivatives, is emerging as a promising strategy for IUAs.

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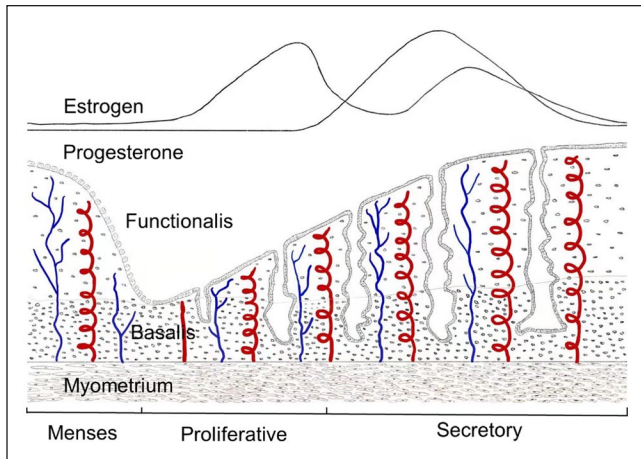Email: zhengshengxia@ustc.edu.cn

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Figure I. Schematic showing that the periodic shedding of human endometrial tissue with the change of estrogen and progesterone during the menstrual cycle. The menstrual cycle is divided into three main phases: menses, proliferative phase, and secretory phase. The basal layer does not change significantly with the menstrual cycle, while the function layer of the human endometrium is periodic shedding.

In this review, we summarize the features of stem cells derived from the endometrial system. More importantly, we review the progress of stem cell therapy for IUAs in animal and human clinical trials.

Stem Cells in Murine Endometrium

The mouse model is crucial to our understanding of endometrial stem cells. A few studies have used label-retention methods to localize stem cells in the endometrium[20]. Bromodeoxyuridine (BrdU) replicate DNA molecules during cell proliferation in place of thymine (T). When BrdU is injected into the mice, all the proliferating cells are labeled, but only the quiescent are identified as label-retaining cells (LRCs)[21,22].

Mouse endometrium stromal stem cells. Through BrdU tracing, Chan and Gargett[20] found that stromal LRCs (6%) mainly reside at the endometrial–myometrial junction near blood vessels and adjacent to the luminal epithelium. Later, in 2007, Cervellóet et al. identified that the stromal LRC population expressed c-Kit (stem cell factor receptor) and octamer-binding transcription factor 4 (OCT-4, pluripotent stem cell marker), also known as POU5F1, 8 to 10 weeks after injection of BrdU. The labeled cells reside primarily in the lower part of the endometrial stroma[23]. A recent study found that 6 weeks after BrdU injection, LRCs primarily resided under the luminal epithelium and expressed CD44+, CD90+, CD140b+, CD146+, and Sca-1+. This study also highlights that LRC responds effectively to physiological stimuli at the onset of uterine involution and returns to the quiescent state after postpartum repair[24]. In a

mouse menstruation model, Yin et al. confirmed that SM22α+ stromal cells, located in the stroma at an early phase, express CD34+KLF4+ markers upon estrogen induction. These cells are transferred to the epithelium during endometrial repair, and this process is called mesenchymal-to-epithelial transition (MET)[25].

Mouse endometrium epithelial stem cells. Chan et al.[26] concluded that epithelial LRCs were mainly located in the luminal epithelium, with few cells observed in the glandular epithelium according to the BrdU method. Huang et al.[27] described a novel transition of stromal cells to epithelial cells during regeneration induced by parturition in 2012. Janzen et al. suggested that CD44+ epithelial cells were mouse epithelial progenitor cell markers, and these cells produced more glandular structures than CD44− cells in immunosuppressed mice. However, CD44+ cells were also expressed in the endometrial stroma[28]. In 2006, Deane et al.[29] used a GFP reporter under the control of the telomerase reverse transcriptase promoter (mTert-GFP) and found that mTert-GFP was expressed by rare luminal and glandular epithelial cells. However, these cells are different from slow-cycling cells with a label-retaining phenotype.

However, BrdU has some limitations in determining stem cell populations because of the length of tracing and the interval of the initial pulse[30]. To solve this problem, several other techniques have been used to trace stem cells. Using a single-cell lineage tracing system, Jin et al. confirmed that in the adult mouse uterus, epithelial stem cells were located at the junction of the glandular epithelium and luminal epithelium. These epithelial stem cells are able to grow both glandular epithelial (GE) and luminal epithelial (LE) efficiently and maintain the homeostasis of the mouse endometrial epithelium[31]. More stringent uterine lineage tracing studies have been performed more recently. In one study, the authors found that Axin2+ cells were located at the base of the gland and were able to regenerate the entire gland[32]. They believed that there were two types of stem cells in the endometrium, a group of short-lived LE progenitor cells and a group of long-lived bipotent stem cells, to maintain stem cell properties and prevent cells from becoming cancerous. In another study, Seishima et al.[33] confirmed that LGR5+ progenitor cells are located at the end of the endometrial glands and maintain uterine gland development after birth.

Stem Cells in Human Endometrium

It was a long time since Prianishnikov proposed that there were stem cells in the deepest basal layer of human endometrium in 1978. These stem cells have the ability to differentiate into endometrial cells, and the ratio of different cells was regulated by hormones produced by the ovaries[34]. Later, Chan et al.[35] first isolated epithelial and stromal stem cells from human endometrium and proved that they have clonogenic activity *in vitro* in 2004. With the development of stem cell
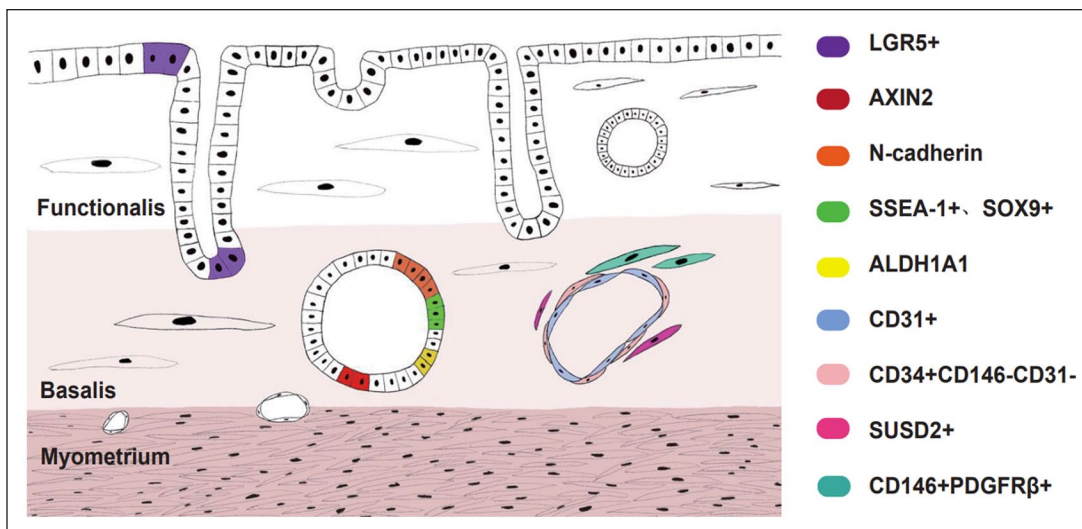
Figure 2. Stem cells in human endometrium and its location. LGR5 are mainly locate in luminal and basalis epithelium. AXIN2+ epithelial cells are locate in basalis of human endometrium. N-cadherin+ epithelial cells are in deep bases of branching gland structures. SSEA-1+ SOX9+ epithelial cells locate in basal layer proximal to N-cadherin+ epithelial progenitor cells. ALDH1A1 co-localize with N-cadherin+ cells in basal glands. CD31+ cells are endothelial stem cells. CD34+ CD146− CD31− cells are perivascular in the adventitia of blood vessels. SUSD2+ eMSCs are also perivascular cells. CD140b+ CD146+ eMSCs are located mainly in both the functionalis and basalis.

research over the years, stem cells in human endometrium have been identified and divided into three types: epithelial stem cells, endothelial stem cells, and endometrial mesenchymal stem cells (MSCs).

Human endometrium stromal stem cells. Endometrium stromal stem cells are similar to other MSCs derived from other human tissues. They are positive for CD105, CD73, CD44, and CD90 and negative for CD45, CD34, CD14, or CD11b[36]. C-kit/CD117 was first identified as an endometrial stem cell marker in 2006. Although C-kit was not detected in the fetal endometrium, it was mainly expressed in the stroma in all other lifetime endometrium samples[37]. Stromal cells positive for two perivascular cell markers, CD146 and platelet-derived growth factor–receptor beta (PDGFR-b), show mesenchymal stem cell (MSC) properties and are located near blood vessels in the human endometrium[38]. Another study identified Sushi domain containing 2+(SUSD2+) (also called W5C5) as a marker for endometrial stromal stem cells. The proliferative activity of SUSD2+ cells was three times higher than that of unsorted endometrial stromal cells and 14.7 times higher than that of W5C5− cells. These cells can also differentiate into osteocytes, adipocytes, and chondrocytes *in vitro* in response to specific induction cues. More importantly, compared with the combination of CD146 and PDGF-Rβ, SUSD2 can be used as a single marker for the extraction of endometrial MSCs[39,40].

Other MSC markers, such as mesenchymal stem cell antigen-1 (MSCA-1), also known as TNAP[41], and Notch1[42], are also found in human endometrial stem cells. The expression of TNAP in endometrial stromal cells was mainly limited to CD146(+) cells[41,43]. Musashi-1, which encodes an RNA-binding protein in neural stem cells[44], was also indicated as an endometrial stromal stem cell marker in 2008. Researchers found that Musashi-1 was highly expressed in the proliferating endometrium, endometriosis, and endometrial carcinoma[45]. Recently, Musashi-1 expression was also found in neonatal endometrium at 12 weeks of gestation, and the number of positive cells decreased with increasing gestational age. When reaching reproductive age, these positive cells were mainly located in stromal cells adjacent to the myometrium[46]. However, no studies have yet successfully isolated Musashi-1+ cells by fluorescence-activated cell sorting (FACS) or magnetic-activated cell sorting (MACS), so its use as an endometrial stem cell marker is controversial. The schematic is listed in Fig. 2.

Human endometrium epithelial stem cells. Endometrial epithelial stem cells are thought to reside in the base of the basal layer glands[30,47]. Although mouse studies indicated that parturition can promote stromal cell transfer to epithelial cells during regeneration, it has not been proven in the human system[27]. In humans, endometrial epithelial stem cells were first identified in 2004 as clonogenic cells marked by EpCAM. EpCAM+ cells showed self-renewal by serially cloning more than three times and proliferated greater than 30 fold during 4 months of culture[35]. Stage-specific embryonic antigen-1 (SSEA-1) has also been identified as a marker for glandular epithelial cells located in the basal layer of the endometrium. SSEA-1+ cells had longer telomeres and a

Table 1. Human Endometrial Stem Cells.

Surface markers	Cell type	Location	Function	Reference
TERT	Epithelial	Glandular	Correlated with epithelial proliferation	[55]
CD326 (EPCAM)	Epithelial	Basalis and functionalis	Endometrial EpCAM(+) epithelial cells own great proliferation abilities	[56]
SOX9	Epithelial	Basalis (46.2–52.3%) functionalis (8–12.1%)	Epithelial repair and glandular regeneration	[47]
SSEA1	Epithelial	Basalis	Epithelial repair and glandular regeneration	[47]
CTNNB1 (β-catenin)	Epithelial	Basalis	Could have stem cell properties	[47]
Notch	Epithelial	Basalis and functionalis	Maintain cells in an undifferentiated state[57]	[57]
CHD2 (N-cadherin)	Epithelial	Basalis	Glandular regeneration and self-renew	[49]
ALDH1AI	Epithelial	Deep glands of basalis	Endometrium regeneration, involved in the pathology of endometriosis	[52]
LRG5	Epithelial	Luminal epithelium and stratum basalis	Correlate with epithelial proliferation	[50]
MSCA-1 (TNAP)	Stromal	Pericyte	High levels in the luminal space of glandular epithelia	[41]
W5C5 (SUSD2)	Stromal	Perivascular, basalis and functionalis	Reconstituting endometrial stromal tissues	[39]
CD146 PDGF-Rb (CD140b)	Stromal	Perivascular location in the basalis (mainly)	Colony-forming ability and multipotency	[38]
NTPDase2	Stromal	Basalis	NTPDase2 was expressed by the SUSD2+ endometrial mesenchymal stem cells	[54]
c-KIT/CD117	Epithelial/ stromal	Mainly in the stroma of the basalis	Persistent from the fetal to the postmenopausal period	[37]
Musashi-1	Epithelial/ stromal	Basalis and functionalis	Progenitor cells in proliferating endometrium	[45]

weaker differentiated phenotype than SSEA− cells, and SSEA+ cells formed more organoids than SSEA− cells[47]. In addition, both SOX9 and β-catenin have been suggested as putative markers of human endometrial epithelial stem cells with SSEA-1[47]. Another putative marker for endometrial epithelial stem cells is N-cadherin, an intercellular adhesion molecule that stabilizes connections between epithelial cells[48]. Immunofluorescence staining showed that N-cadherin was mainly located at the basal layer near the myometrium, and N-cadherin+ epithelial cells had stronger colony formation ability *in vitro* than N-cadherin− epithelial cells[49].

A few other genes have been proposed as endometrial epithelial stem cell markers; however, the results are not convincing due to the lack of functional analysis. In one study, the researchers used *in situ* hybridization (ISH), qPCR, and a systems biology approach to study the expression of leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5), a marker for the intestinal epithelium and hair follicle stem cells, in human endometrium. They found that LGR5 was more dominantly expressed in the luminal epithelium than in other epithelial compartments in the healthy human endometrium[50]. However, due to the poor quality of LGR5 antibodies, further functional analysis could not be conducted. In another study, Ma et al. found that aldehyde dehydrogenase 1 isoform (ALDH1A1), a cancer stem cell marker[51], was highly expressed in the epithelium of basalis in the human endometrium and colocalized with N-cadherin in the glands of stratum basalis[52].

Another putative marker is ectonucleoside triphosphate diphosphohydrolase-2 (NTPDase2), a cell surface enzyme that can catalyze the dephosphorylation of ATP[53]. A recent study found that NTPDase2 is present in the surrounding basal glands but absent in functional glands. They proposed that NTPDase2 may be related to endometrial epithelial stem cell storage[54]. All markers and details are listed in Table 1.

Endometrial-Derived Stem Cells for IUA Treatment

Animal models for IUA. A suitable animal model that recapitulates the pathology of IUA is crucial for testing novel regenerative therapies in preclinical phases. Till now, mice, rats, rabbits, and nonhuman primates have been used to model IUA. Owing to the size of the animals and the cost, rats are most often used for research and preclinical studies. Uterine histology, morphology, and pregnancy outcome are usually assessed to evaluate whether the models mimic the physiology and pathology of the disease[58,59].

Endometrial injury was induced by physical damage, chemical damage, or a combination of both[60]. Physical injury by surgical blades is often performed on rats to mimic the process of human endometrial curettage IUAs[61]. This process is always accompanied by side effects, such as abdominal adhesion. In addition, the process is laborious and depends highly on the surgical technique, which makes

consistency a major issue in interpreting endpoint results. Chemical damage by ethanol is another well-used method to mimic IUAs[62,63]. However, the retention time and the dosage of ethanol are difficult to determine to avoid excessive or insufficient endometrial damage[64]. Trichloroacetic acid (TCA), a chemical agent that causes chemical burns to tissue once in contact, is also used for IUAs models. TCA is used clinically to treat patients with dysfunctional uterine bleeding (DUB) through endometrial ablation[65]. However, TCA is reported to be carcinogenic according to the World Health Organization's International Agency[66]. A comprehensive comparison of various rat IUAs models was conducted by one group, and they concluded that compared with heat stripping, mechanical injury, and the combination of mechanical injury and infection (dual-injury), ethanol injection was the best based on histological and immunohistochemical analysis[67]. More recently, a study established an IUAs model by surgical abortion and curettage in pregnant rats. They claim that this process mimics abortion curettage or postpartum curettage in humans and that the pathology is more comparable to human IUAs[68].

Despite numerous improvements in animal models, there is wide variation in procedures, timing, endpoints, and assessment criteria, resulting in inconsistent results between studies and inaccurate conclusions for efficacy tests. To avoid future failure in human clinical trials, appropriate assessment plans with validated methods, statistically prejustified sample sizes, timing, endpoints, and evaluation methods should be developed for preclinical animal studies of stem cell therapy.

Menstrual blood–derived stem cells for IUA treatment. Menstrual blood–derived stem cells (MenSCs) were first identified in 2007 and found to express CD9, CD29, CD41a, CD44, CD59, CD73, CD90, and CD105, which are also expressed by MSCs from other tissues. Compared to MSCs, MenSCs have several advantages over MSCs derived from other tissues for stem cell therapy[69]. First, MenSCs can be easily obtained with noninvasive operation and no ethical concerns[70,71]. Second, MenSCs are able to proliferate rapidly and remain karyotype stable after 68 generations[72]. Finally, MenSCs express high level of matrix metalloproteases, which is beneficial for tissue repair[69]. So far, animal experiments have shown that MenSCs have therapeutic effects on a variety of diseases, including type 1 diabetes[73], cardiac diseases[74], cartilage damage[75], osteochondral defects[76], premature ovarian failure[77], and liver disease[78,79].

MenSCs can differentiate into endometrium cells with growth factor-β (TGF-β), epidermal growth factor (EGF), platelet-derived growth factor BB (PDGF-BB) *in vitro*. Thus, they are supposed to contribute to endometrium growth and recovery of fertility[72]. Animal experiments in rats confirmed that MenSCs was indeed beneficial to the recovery of IUA, and the effect is better when culture MenSCs with FGF-2[80]. Transplantation of MenSCs with platelet-rich plasma (PRP), a platelet concentrate product with rich growth factors and proteins, improves uterine proliferation and endometrium damage repair in IUA rats[81]. Based on these promising results, MenSCs-based treatment are proposed as a personalized strategy for reproductive therapy.

Human clinical trials. As stem cell therapy becomes more standardized and promising, an increasing number of clinical trials of stem cell treatments for IUAs are being conducted worldwide. For example, researchers have used autologous MenSCs to treat IUAs, in which endometrial thickness increased in all patients, and three of seven eventually became pregnant[82]. In another study, the researchers adopted UC-MSC+ collagen scaffolds to treat IUAs. The endometrial thickness of all patients who accepted the treatment was increased, and 10 of 26 achieved successful pregnancy[83]. Similar clinical trials to improve the thickness of IUAs patients included amnion[84] and b-FGF[85], and both obtained promising results. To date, a great number of clinical trials have been conducted to treat infertility in women caused by endometrial factors, and details are listed in Table 2.

All clinical trials of MSC therapy are interventional trials. Most patients recruited were women of normal reproductive age with a history of uterine adhesions or thin endometrium and no history of human chorionic gonadotrophin (HCG) use. Various sources of cells, different injection methods, and combinations with different materials and hormonal supports have been applied in clinical trials. MSCs come from a wide range of sources, such as umbilical cord, adipose, amniotic and bone marrow. The effects of MSC therapy can be measured in many ways, such as endometrial thickness, changes in menstrual volume, reduction of intrauterine scar area, implantation rate, and pregnancy rate before and after treatment. In all clinical trials that we counted, half of the trials were over and had good results. In addition, in all of the clinical trials, only autologous BMSC transplantation was in phase 4, and the remaining trials were in phase 1 and phase 2. These results indicate that autologous BMSCs have great advantages in the treatment of IUAs, and autologous stem cell transplantation will be the first choice of stem cell therapy in the future.

Conclusion

Great progress has been made in stem cell therapy with the rapid development of stem cell research. Since the first *in vitro* fertilization (IVF) baby was born in 1978 in the United Kingdom[86], IVF has made tremendous progress worldwide in the past 30 years. However, female infertility includes not only the problem of seeds (embryo) but also the problems of soil (human endometrium). Stem cells are essential for the maintenance of normal endometrial function and morphology. Therefore, transplantation of stem cells is considered a promising therapeutic strategy to promote endometrial repair or regeneration in IUAs patients. Although many preclinical and clinical studies have shown the potential therapeutic effect of MSCs derived from either

Table 2. The List of Some Clinical Trials Using Mesenchymal Stem Cells to Treat Intrauterine Adhesions (or Thin Endometrium, Asherman's Syndrome). All Data Come From: Home - ClinicalTrials.gov.

Pathological condition	Cell therapy	Estimated enrollment	Intervention/ treatment	Cliniclatrials. gov identifier	Outcome measures
Infertile women with severe intrauterine adhesions or endometrial dysplasia	Collagen scaffold loaded with autologous BMSCs	30 participants	Autologous bone marrow stem cells	NCT02204358	Evaluation of the reduction of intrauterine scar area, the change of intrauterine adhesion, endometrial thickness, and the menstrual blood volume
Women with intrauterine adhesion	UC-MSCs loaded in collagen scaffold	18 participants	UCMSC combined with collagen scaffold	NCT03724617	Evaluation of the change of endometrial thickness, pregnancy rate, live birth rate, abortion rate
Women with scarring and adhesions	AMSCs and a biodegradable carrier	10 participants	Autologous mesenchymal stem cells	NCT04432467	Evaluation of the number of cured patients, and patients with treatment-related adverse events
Women infertility with severe refractory Asherman's syndrome	hAESCs	50 participants	hAESCs-based therapy	NCT03223454	Evaluation of menstrual blood volume, Endometrial thickness, Uterine volume, Ongoing pregnancy rate
Women with severe refractory Asherman's syndrome	hAESCs	20 participants	hAESCs-based therapy	NCT03381807	Evaluation of the changes of Endometrial thickness, Menstrual blood volume, pregnancy rate
Infertility women with Intrauterine adhesions	UCMSCs	26 participants	UCMSC-based therapy	NCT02313415	Evaluation of live birth rate, endometrial thickness, reduction of intrauterine adhesion, change of menstrual blood volume
Patients with Asherman's Syndrome	CD133+ autologous BMSCs	16 participants	Bone marrow CD133+ stem cell transplantation	NCT02144987	Evaluation of Live-birth rate, Ongoing pregnancy rate, Implantation Rate, Endometrial thickness prior to the treatment
Asherman Syndrome Endometrium Recurrent Implantation Failure	Autologous BMSCs	30 participants	BMSCs-based therapy	NCT05343572	Evaluation of endometrial thickness and implantation rates

AMSCs: adipose tissue derived mesenchymal stem cells; BMSCs: bone marrow stem cells; hAESCs: Human amniotic epithelial stem cells; UCMSC: umbilical cord mesenchymal stem cells.

endometrial or nonendometrial tissues on IUAs, improvements should be made on several issues before we develop a novel stem cell therapy with high efficacy and safety: (1) a thorough understanding of adult stem cells and their regulatory mechanisms in the human endometrium; (2) reliable animal models that mimic human IUAs with good predictability of clinical outcomes; and (3) Good manufacturing practices (GMP) regulations on the isolation, expansion, and transplantation of stem cells for clinical application. We believe that in the near future, stem cell therapy will become one of the most effective therapies for a variety of diseases, including IUAs.

Author Contributions

Kai Chen: Drafted and edited the manuscript.
Shengxia Zheng: Concept of manuscript and provided advice about the content.
Fang Fang: Concept and design of manuscript, edited the manuscript, and final approval of the manuscript.

Availability of Data and Materials

Not applicable.

Ethical Approval

Not applicable.

Statement of Human and Animal Rights

This study did not involve any human or animal subjects.

Statement of Informed Consent

Not applicable.

Declaration of Conflicting Interests

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