Bone marrow mesenchymal stem cells could acquire the phenotypes of epithelial cells and accelerate vaginal reconstruction combined with small intestinal submucosa

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Abstract

Grafting material for vaginal reconstruction commonly includes the bowel, peritoneum, skin, and amniotic membrane. Bone marrow mesenchymal stem cells (MSCs) have the potential of multilineage differentiation into a variety of cells and have been widely explored in tissue engineering. In the current study, we examined whether MSCs could be differentiated to vaginal epithelial cells (VECs) upon co-culturing with VECs. We also examined whether Wnt/β-catenin signaling pathway is implicated in such differentiation. Co-culture of MSCs with VECs using a transwell insert system (with no direct contact) induced the expression of VECs marker AE1/AE3 in MSCs. MSCs combined with small intestinal submucosa (SIS) scaffold were implanted in place of the native vagina in rats to observe the implications for vaginal reconstruction in vivo. Anatomic repair of neovagina was assessed by histological staining for H/E and Masson’s Trichrome. GSK-3β and β-catenin, main members of Wnt/β-catenin signaling pathway, in MSCs were increased upon co-culturing with VECs. Exposure of co-cultured MSCs to a Wnt/β-catenin signaling activator, lithium chloride (LiCl, 20 μM) increased phosphorylated GSK-3β and β-catenin and enhanced expression of AE1/AE3. In vivo-grafted cells displayed significant matrix infiltration and expressed epithelial markers in neovagina. These findings suggest that MSCs could acquire the phenotype of VECs when co-cultured with VECs, possibly via activation of Wnt/β-catenin signaling. MSCs provide an alternative cell source for potential use in vaginal tissue engineering.

Keywords: cell differentiation; epithelial cells; mesenchymal stem cells; vagina tissue engineering; Wnt signaling pathway

Introduction

Vaginal reconstruction is a treatment option for women with congenital absence of the vagina, some women receiving radical treatment for malignant tumors of the vagina and surrounding structures, as well as gender reassignment surgery (Özkan et al., 2011). The grafting material is often autologous but non-vaginal in origin, such as the bowel or peritoneum (Panici et al., 2011). The reconstructed vagina is often not ideal in durability, function, and aesthetic appearance (Idrees et al., 2009). Other tissue sources, including the skin, trachea, and urethral tissue (Raya-Rivera et al., 2011) are also considered (Badylak et al., 2012). Animal studies have indicated that native vaginal tissue is preferable for reconstruction (De Filippo et al., 2008). For clinical practice, unfortunately, native vaginal tissue is limited or unavailable at all in some cases.

Bone marrow mesenchymal stem cells (MSCs) have the potential for extensive self-renewal and multi-lineage differentiation (Aldahmash et al., 2012). MSCs could develop into a variety of cells, including neural, vascular, hepatic, pancreatic, and epidermal cells. Many in vitro experiments
have shown that MSCs can differentiate into cells of epithelial lineages, such as corneal epithelial cells, airway epithelial cells, and urothelial cells (Gu et al., 2009; Tian et al., 2010). Mechanistically, the Wnts signaling pathway plays an important role in the self-renewal and differentiation of MSCs, including osteogenesis, adipogenesis, chondrogenesis, and epithelial differentiation (Augello and De Bari, 2010). Wnts bind to the receptor protein Frizzled (Fzd) and LRP5/6 and suppress the phosphorylation activity of glycogen synthase kinase 3β (GSK-3β), causing dissociation of β-catenin from the adenomatous polyposis coli (APC)-axin-GSK-3β complex (Ling et al., 2009). β-catenin is accumulated in the nucleus where it associates with TCF/Lef transcription factors and induces transcription of target genes (Ling et al., 2009), leading to self-renewal and differentiation of MSCs. The possibility of epithelial differentiation of MSCs induced by VECs has not been investigated so far. In the current study, we examined whether cultured bone marrow MSCs could differentiate into epithelial-like cells upon coculturing with VECs in vitro. Possible role of Wnt/β-catenin signaling in this process was also investigated. Moreover, role of MSCs combined with small intestinal submucosa (SIS) in vaginal reconstruction in vivo was explored.

Materials and methods

MSCs preparation

All relevant protocols were approved by the Institutional Animal Care and Use Committee of Hebei Medical University. Female Sprague–Dawley rats weighing 90–110 g were sacrificed by cervical dislocation. Bone marrow from the femurs and tibias was flushed out by using DMEM/F12 medium (Hyclone, Thermo Scientific, Beijing, China) under aseptic conditions, filtered through a 70-μm sieve, and centrifuged at 200 g for 5 min. The pellet was re-suspended in DMEM/F12 containing 10% fetal bovine serum (FBS; Hyclone, Thermo Scientific), 2 mM glutamine, 1% penicillin, and streptomycin (Hyclone, Thermo Scientific). Cells were plated at a density of 1.5 × 10^5 cells/cm² and cultured in a humidified atmosphere containing 95% air and 5% CO₂ at 37°C. Non-adenherent cells were discarded after 48 h. The culture medium was replenished every 3–4 days and passaged at a density of 1 × 10⁴ cells/cm² upon 70–80% confluency with 0.25% trypsin/0.02% EDTA (Sigma–Aldrich, St. Louis, MO). Cells of the third to fifth passage were used for all experiments.

Flow cytometry (FCM) verification of MSCs

Cells were stained for the following markers: CD34, CD45, CD29, and CD90. Briefly, 1 × 10^5 – 1 × 10^6 cells in 100 μL of cold PBS were incubated with PE-conjugated anti-rat CD34 (Santa Cruz Biotechnology, Santa Cruz, CA), FITC-conjugated anti-rat CD45 (Biolegend, San Diego, CA), PE-conjugated anti-rat CD29 (Biolegend), or PE-conjugated anti-rat CD90 (Biolegend), and all of the following isotype antibodies: PE-mouse IgG1 isotype control antibody, FITC-mouse IgG1, κ isotype control antibody, and PE-Armenian hamster IgG isotype control antibody (Biolegend) for 30 min in the dark. After extensive washing, cells were analyzed with an Epics-XL flow cytometer (Becton Dickinson, San Jose, CA) equipped with Expo 32 ADC software (Becton Dickinson).

Potential for multi-lineage differentiation of MSCs

The potential for multi-lineage differentiation was tested using a standard protocol (Soleimani and Nadri, 2009). For osteoblast differentiation, cells were seeded at a density of 1 × 10^4/cm² and incubated in DMEM/F12 containing 10% FBS, 0.1 μM dexamethasone, 50 μM ascorbate-2-phosphate, and 10 mM β-glycerophosphate (Sigma–Aldrich). The medium was replenished twice per week. After 14 days of culture, alkaline phosphatase (ALP) activity of the cells was assessed using an ALP immunocytochemical kit (Jiancheng, Nanjing, China). After additional 14 days, the cells were stained with Alizarin Red (Amresco, Solon, OH) to evaluate the mineralization capacity. For adipogenic differentiation, cells were seeded at a density of 1 × 10^4/cm² and incubated in DMEM/F12 containing 10% FBS, 1 μM dexamethasone, 0.5 mM IBMX, and 10 μg/mL insulin (all from Sigma–Aldrich). The medium was replenished twice per week. For assessment of lipid accumulation, cells were fixed with 4% paraformaldehyde and incubated in 0.5% Oil Red O (Amresco) working solution for 30 min at room temperature. Cells containing stained vacuole(s) were considered positive. For chondrogenic differentiation, 2.5 × 10^3 MSCs were cultured in chondrogenic induction medium (Cyagen, Guangdong, China) supplemented with 10 ng/mL of transforming growth factor β3, 0.1 μM dexamethasone, and 50 μg/mL ascorbic acid for 3 weeks. Cells were fixed with formalin. Chondrocyte nodules were paraffin embedded and stained for glycosaminoglycans with 1% toluidine blue for 30 min. MSCs that were not induced were processed in parallel as a negative control for three experiments.

Vaginal epithelium cells

Female Sprague–Dawley rats weighing 250–300 g served as sources of vaginal tissue. The vaginal tissues were dissected out, cleaned of connective tissues, minced into small pieces (10 mm × 5 mm), rinsed three times with PBS containing penicillin (100 U/mL) and streptomycin (100 mg/mL), and digested with dispase II (Sigma–Aldrich; 1.2 U/mL) overnight at 4°C. VECs were isolated by three rounds of enzymatic digestion with 0.25% trypsin/0.02 EDTA for 10 min each at 37°C. The cell suspension was centrifuged at
200g for 5 min. The pellet was re-suspended in keratinocyte growth medium (KGM-Gold, Lonza, Walkersville, MD) with growth supplement (Lonza), and seeded at a density of $1.0 \times 10^7$ cells/cm$^2$, and maintained in KGM with medium changes every 48–72 h. The cells were sub-cultured upon 80–90% confluency. Epithelial cell phenotypes were confirmed by morphology and immunohistochemical staining with antibodies to pan cytokeratins (AE1/AE3) (Santa Cruz).

**Differentiation of MSCs in vitro**

For differentiation, MSCs (2,500–3,000 cells/cm$^2$) were co-cultured with vaginal epithelial cells (VECs) using cell culture transwell inserts (0.4-μm pore, 4.5 cm$^2$, Corning, MA) for 14 days. Fibronectin/collagen type I (FN/C, Sigma–Aldrich)-coated inserts were dehydrated, loaded with VECs in KGM, and incubated for 48 h. Then, MSCs were dispensed into the culture well and incubated for 24 h. At the end of the incubation period, VECs in the top chamber and MSCs in the bottom chamber were washed, and 3 mL of medium was added to the culture wells. The culture system was assembled by morphology and immunohistochemical staining with antibodies to pan cytokeratins (AE1/AE3) (Santa Cruz).

**Immunocytochemical staining**

Cells were fixed with 4% paraformaldehyde for 20 min at room temperature, permeabilized with 0.3% Triton X-100 for 10 min. Endogenous peroxidase activity was quenched with 0.1% hydrogen peroxide for 10 min. After blocking with 5% goat serum for 1 h at 37°C, cells were incubated with pan-cytokeratin (AE1/AE3) monoclonal antibodies (1:50 dilution) overnight at 4°C. The secondary antibody was a horseradish-peroxidase-conjugated goat anti-mouse IgG (ZSGB-BIO, Beijing, China). Color reaction was carried out using a DAB kit (AbsoluxDx, Abgent, Inc., San Diego, CA). After counterstaining with hematoxylin, the cells were examined under a light microscope. VECs and MSCs cultured alone were used as controls.

**Western blot assay**

Cells were extracted in a buffer containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM PMSF, 1 mM EDTA, 1% Triton X-100, and 0.1% SDS for 30 min on ice. The extract was centrifuged at 10,800g for 30 min. Proteins in the supernatant were separated on 8% SDS–PAGE gels, and electroblotted onto polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA). The membrane was blocked with 5% non-fat dry milk in PBS for 1 h. The blots were incubated with antipan-cytokeratins (AE1/AE3), β-catenin, GSK-3β, pi-GSK-3β, TCF-3, or GAPDH antibodies overnight at 4°C, followed by incubation with a fluorescent labeled IRDye 800 secondary antibody in the dark for 2 h at room temperature. The analysis was carried out using LI-COR Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE). Protein concentration was examined using Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE).

**Construction of neovagina**

Institutional Animal Care and Use Committee of Hebei Medical University approved the animal surgery procedures for this study. Porcine SIS was manufactured following the protocol of Luo et al. (2011). MSCs were labeled with 10 μmol/L bromodeoxyuridine (5-bromo-2-deoxyuridine, BrdU) (Solarbio, Beijing, China), and then seeded on 1.5 cm $\times$ 1.8 cm wetted SIS. After 7 days of culturing, scaffold was constructed by rolling MSCs seeded SIS on a rubber hose. MSCs were in the outer surface. Scaffolds were implanted in place of the native vagina in 10 female rats. Upper end of the scaffold was sutured to the lateral side of cervix, and the other end was sutured together with the vaginal opening cut edge. Unseeded control scaffolds were implanted into 10 female rats. All grafts were retrieved at 20 and 30 days after surgery. The implanted grafts were then fixed in 10% buffered formalin, embedded in paraffin, and assessed with hematoxylin and eosin (HE), Masson’s trichrome (MTC), and immunohistochemical (IHC) staining for BrdU and AE1/AE3.

**Statistical analysis**

All experiments had been independently replicated at least three times, and three independent observations that were used for the statistical analysis. Target protein was normalized by GAPDH, and expressed as mean ± standard error (SE). Data were analyzed with Student’s t-test between two groups, using SAS 9.1 software (SAS Institute, Inc., Cary, NC). Significance was set at $P < 0.05$.

**Results**

**Biological properties of MSCs**

The bone marrow cells began to stretch after 2 days of culture. Distinct colonies of fibroblastic cells formed in various sizes after 7–10 days of culture. After three
subcultures, the cells had a spiral whorl-like outlook. Flow cytometry analysis of the cells revealed high expression of CD29 and CD90, but not CD45 and CD34.

Upon osteogenic induction, ALP activity increased after 14 days of culture, and mineralized nodules formed after 4 weeks of induction. For adipogenic differentiation, intracellular Oil-red-O stained lipids appeared after 2 weeks of culture. For chondrogenic differentiation, blue staining indicates synthesis of proteoglycans by chondrocytes after 3 weeks of culture (Supplemental Figure).

Differentiation of MSCs into epithelial cells

MSCs displayed spindle or polygonal shape upon co-culture with VECs for 7 (Figure 1b) and 14 days (Figure 1c). Immunocytochemical staining revealed expression of the epithelial marker AE1/AE3 in MSCs upon co-culture with VECs (Figure 1f, g).

Western blot analysis revealed a time-dependent increase in the expression of AE1/AE3 in induced MSCs within a 14-day time frame (Figure 1i). Notably, significantly higher level of AE1/AE3 was detected on 14th day than on 7th day (Figure 1i).

Wnt/β-catenin signaling during epithelial differentiation of MSCs

Co-culture with VECs increased the protein level of β-catenin, pi-GSK-3β, and TCF-3 and decreased GSK-3β in MSCs (Figure 2a, b). The changes were more prominent on 14th day than on 7th day of co-culture, with TCF-3 as an exception: TCF-3 was lower on 14th day than on 7th day. Phosphorylation GSK-3β in induced MSCs was opposite to that with total GSK-3β (Figure 2a, b).

Activation of Wnt/β-catenin signaling promotes epithelial differentiation of MSCs

In the co-culture of MSCs and VECs, LiCl increased the protein level of β-catenin and TCF-3 and decreased GSK-3β (Figure 2c, d). DKK-1 decreased the protein level of β-catenin and TCF-3 and increased GSK-3β.

Figure 1 Co-culture of MSCs with vaginal epithelial cells (VECs) induced morphological changes and expression of the VEC marker AE1/AE3 in MSCs. Phase-contrast images of MSCs differentiating into epithelial cells on (a) day 0, (b) day 7, (c) day 14, and (d) VECs on 0.4 μm transwell insert. Scale bars represent 70 μm. Immunocytochemical staining of AE1/AE3-positive MSCs with VEC-like morphology on (f) day 7 and (g) day 14. MSCs (e) and VECs (h) were used as negative and positive controls. Scale bars represent 37.5 μm. i, j: Western blot detection of AE1/AE3 protein expression in co-cultured MSCs with VECs on days 0, 7, 14, and VECs. GAPDH served as an internal control. (**P < 0.01 vs. control; §P < 0.05 14 days vs. 7 days; t-test; the error bars indicate the standard error of the mean from three experiments).
**Figure 2** a,b: MSCs co-cultured with VECs induced changes in the protein expression of several important members of the Wnt/β-catenin signaling pathway in MSCs. Western blot analysis of protein levels of β-catenin, GSK-3β, pi-GSK-3β, and TCF-3 in MSCs co-cultured with VECs on days 0, 7, 14, and in VECs. (*P < 0.05 vs. control; **P < 0.01 vs. control; §P < 0.05 14 days vs. 7 days; t test; the error bars indicate the standard error of the mean from three experiments). After 7 days of co-culture, epithelial differentiation capacities of MSCs were assessed by treatments with 20 μM LiCl and 20 ng/mL DKK-1. c,d: Western blot detection of the protein levels of β-catenin, GSK-3β, TCF-3 in MSCs treated with LiCl and DKK-1 and subjected to 7 days of co-culture with VECs. e,f: Western blot analysis of protein levels of AE1/AE3 in MSCs treated with LiCl and DKK-1 and subjected to 7 days of co-culture with VECs. GAPDH served as an internal control. (*P < 0.05 vs. co-control; **P < 0.01 vs. co-control, t-test; the error bars indicate the standard error of the mean from three experiments).
LiCl increased AE1/AE3 expression in MSCs upon coculturing with VECs was increased by LiCl and decreased by DKK-1 (Figure 2e, f).

Epithelial differentiation of MSCs in vivo

Gross examination of unseeded and seeded SISs showed patent tissue 20 days after surgery. Histologic analysis showed that new VECs grown from vaginal orifice upward to the upper end and spread only half of the full vaginal wall in unseeded SIS 20 days after implantation (Figure 3a, b). While MSCs seeded SIS construct was beginning to form typical multilayered architecture of a normal vagina (Figure 3p, q) 20 days after implantation (Figure 3g, h).

Thirty days after implantation, both unseeded and seeded SISs showed complete epithelium (Figure 3d, j). The tissue structure of seeded SIS closely resembled that of a normal vagina with defined layers of collagen (green), blood vessels, and an invaginated epithelium (Figure 3k). Immunohistochemical staining for cytokeratins AE1/AE3, a marker of the epithelial cell lineage, showed that a layer of epithelial cells was apparent by 20 days (Figure 3i) becoming clearly defined and developed by 30 days (Figure 3l) and resembling that of a native vagina (Figure 3r). Immunohistochemical staining for Brdu indicated that BrdU-positive cells existed in vaginal epithelium. While BrdU-positive cells just existed in the surface of new vagina and had not formed epithelial cells where no epithelium covering (Figure 3n, o).

Discussion

In the current study, we demonstrated that bone marrow MSCs could differentiate into VEC-like cells upon co-culture with VECs, likely via the activation of the Wnt/β-catenin signaling pathway. The co-culture system used in the current study used a semi-permeable membrane that avoided contact and allowed paracrine function between MSCs with VECs, yet excluded the possibility of cell fusion and endocytosis.

The Wnt/β-catenin signaling pathway is a key regulator of stem cell self-renewal and differentiation (Saraswati et al., 2012). The effects of Wnt/β-catenin signaling on differentiation is context-dependent. In a study of the role of Wnt/β-catenin in the cementoblast/osteoblast differentiation of rat dental follicle cells (DFCs), LiCl enhanced β-catenin/T-cell factor luciferase activity and alkaline phosphatase activity, suggesting that Wnt/β-catenin positively regulates the cementoblast/osteoblast differentiation of the DFCs (Du et al., 2012b). Another study showed that aged MSCs treated...
with LiCl recovered the lost capacity for myogenic differentiation (Brunt et al., 2012). A positive role for Wnt/β-catenin signaling in the differentiation of MSCs has been also demonstrated in other studies (Fathke et al., 2006). However, in a study in pig blastocysts, the rate of blastocyst hatching was increased by the Wnt/β-catenin inhibitor DKK-1 and decreased by LiCl (Lim et al., 2013). Similar negative effects of Wnt/β-catenin signaling were also shown in osteogenic (Boland et al., 2004; Li et al., 2011) and hepatocyte (Ke et al., 2008) MSC differentiation. Some studies even suggested that Wnt signaling could decide the fate of MSCs differentiation into chondrocyte versus osteoblast (Day et al., 2005) and adipocyte versus osteoblast (Cawthorn et al., 2012).

For MSC differentiation into epithelial cell lineages, Wnt/β-catenin signaling also plays a critical role (Popov et al., 2007; Rabbani et al., 2011). GSK-3β could down-regulate the Wnt/β-catenin signaling by phosphorylation and inactivation of β-catenin (Ling et al., 2009). However, the role of canonical Wnt signaling in the epithelial differentiation of MSCs is controversial. In a study by Wang et al. (2009), rat airway epithelial cells served as a source of epithelium of in vitro coculture system. The activation of β-catenin induced by LiCl prevents epithelial differentiation of MSCs, whereas the inhibition of β-catenin by DKK-1 promotes the differentiation of MSCs into epithelial cells. Cell type and culture microenvironment also contributes to the differing findings on role of Wnt/β-catenin signaling in MSC epithelial differentiation. In the current study, the Wnt/β-catenin signaling pathway was activated in MSCs upon co-culturing with VECs, as reflected by the increase of phosphorylated GSK-3β and the accumulation of β-catenin. The expression of the VECs marker AE1/AE3 was increased when the Wnt/β-catenin signaling was stimulated with LiCl and decreased when Wnt/β-catenin signaling was inhibited by DKK-1.

SIS is a natural biomaterial derived from the small intestine of vertebrates, usually from swine. SIS consists of collagen, proteoglycan glycosaminoglycan, glycoprotein, and growth factors, all of which together make SIS an excellent choice as an ideally implanted material for tissue engineering and clinical applications (Shi and Ronfard, 2013). Ding et al. (2014) shared positive experiences of combined laparoscopic and vaginal cervicovaginal reconstruction using an SIS graft in eight patients, with successful reconstruction and no complications, cervical stenosis, or vaginal stenosis. All the patients showed resumption of menstruation.

A cellular component may be required for better reconstruction. SIS seeded with cultured homologous smooth muscle cells were used to repair the bladder of female dogs and were found could improve the quality of tissue repair, and consequently decrease the potential complications inherent to acellular SIS (Rossetto et al., 2013). In a study of New Zealand male rabbits (Greca et al., 2013), the implanted graft proved superior to transverse coloplasty regarding the increase in distal colon diameter. Remarkable regeneration, marked fibroplasia, and epithelium coverage occurred throughout the graft on the 30th postoperative day. The graft of SIS combined with autologous oral mucosal epithelial cells promotes re-epithelialization and muscular regeneration in a cervical esophageal defects model of male beagle dogs (Wei et al., 2009). As far as tracheal reconstruction was concerned, SIS combined MSCs showed minimal tracheal stenosis, minimal infiltration of the inflammatory cells, and granulation tissue formation, and regeneration of pseudostratified columnar epithelium compared to the untreated group and SIS group in rabbits (Du et al., 2012a).

Despite advances in wound closure techniques and devices, there is still a critical need for new methods of enhancing the healing process to achieve optimal outcomes. Stem cell therapy has emerged as a new approach to accelerate wound healing. Nie et al. (2011) locally administered adipose-derived stem cells (ASCs) to an excisional wound healing model in rat and found that ASC therapy could accelerate wound healing through epithelialization differentiation and vasculogenesis and might represent a novel therapeutic approach in cutaneous wounds.

Our work find thatMSCs can accelerate formation of collagen, blood vessels, and epithelium in vaginal reconstruction with MSCs seeded SIS. A key question in tissue engineering is whether the cells that compose the mature neoorgan are derived from the original cell-seeding or whether a substantial number of cells migrate in from the surrounding tissue. To answer this question for the neovaginal constructs, epithelial cells were labeled with BrdU. BrdU is a pyrimidine analogue of thymidine, selectively incorporated into cell DNA at the S phase of the cell cycle. The application of monoclonal antibodies which react specifically with BrdU for detection of labeling cells. In 20 days post implantation, labeled cells were easily detected (Figure 3n). The labeled VECs were found in a well-organized layer at the surface (brown), coincident with regions that showed expression of cytokeratins (Figure 3o). These indicate that most of epithelial and smooth muscle cell populations present in the regenerated vagina derive from the original seeded MSCs. While Brdu-positive cells just existed in the surface of new vagina and had not formed regular epithelial cells where no epithelium covering. This indicated that native VECs or local microenvironment may take part in differentiation of MSCs to epithelial cells.

Analysis at later stages with this technique is not possible because of the dilution of the markers. A more accurate, long-term analysis, in an autologous situation, will require permanent cell marking such as lentiviral transduction. In addition to the histological studies, optimal functionality in an engineered vagina requires biomechanical properties of grafts. Due to financial limitation, we failed to
assess this part. Expression of nerve fibers, collagens, and elastin are also needed to be detected in following research. Since no specific markers founded in VECs, AE1/AE3 were used as the only marker demonstrating epithelium. A group of markers may improve verification, such as estrogen receptor, and progestogen receptor. But these markers still need to be confirmed in specific lab animals.

Conclusions

In conclusion, our findings demonstrated that bone marrow MSCs could be differentiated into VEC-like cells upon coculturing with VECs. Activation of Wnt/β-catenin signaling enhanced this process. MSCs can accelerate vaginal reconstruction and the effect may achieve through MSCs differentiation. Differentiation of MSCs into VECs could be induced by the microenvironment of VECs. The use of MSCs combined with SIS engineered vaginal tissue represents an attractive option for surgical reconstruction of vaginal pathological disorders in women.

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Conflict of interest

There is no conflict of interest.

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Supporting Information
Additional supporting information may be found in the online version of this article at the publisher’s web-site.

Figure S1. Biological properties of MSCs.