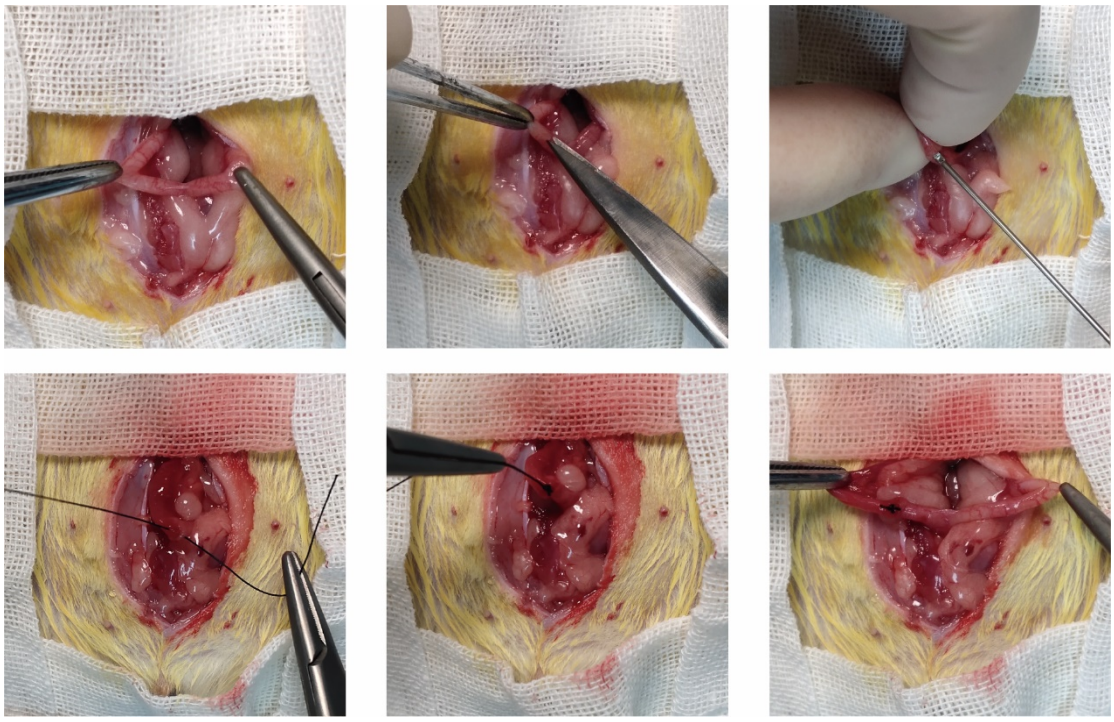
Supplementary Information

Effective repair of endometrial injury in rats using enzyme cross-linked gelatin hydrogel with human menstrual blood-derived stem cells

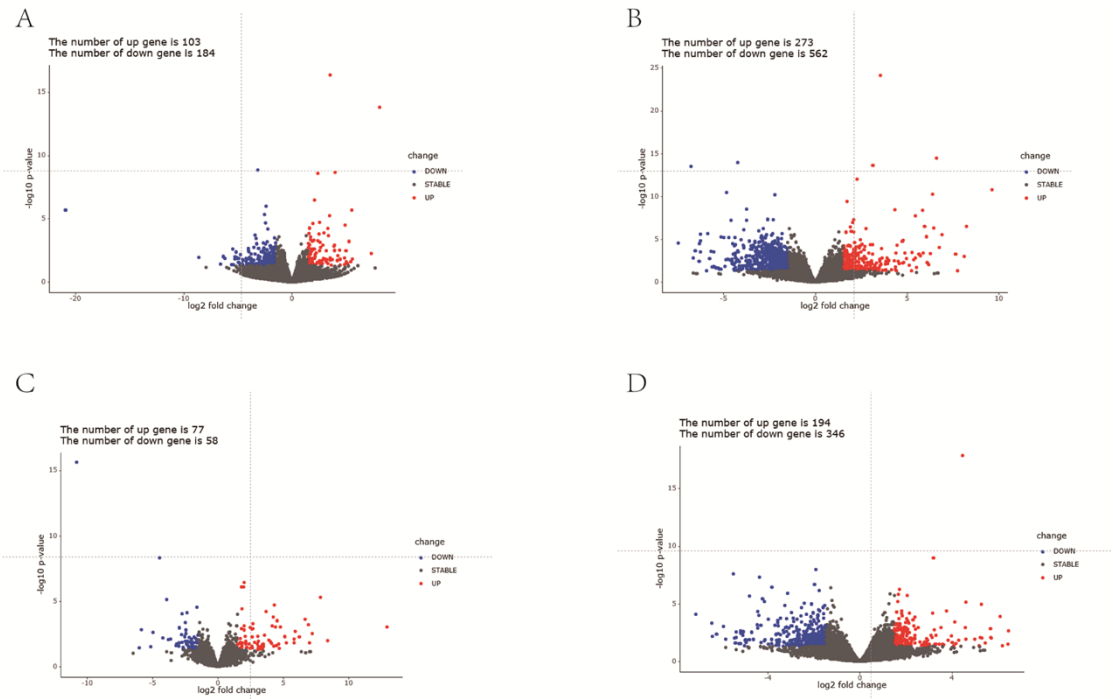
Supplementary Figure 1. Estrous cycle of rats.

The physiological cycle of rats consists of four stages including the diestrus stage, proestrus stage, estrus stage, and metestrus stage. During the diestrus stage, the majority of cells are leukocytes with a small number of nucleated epithelial cells present. In the proestrus stage, the majority of cells are nucleated epithelial cells with a few keratinized epithelial cells lacking nuclei. The estrus stage is characterized by the presence of a large number of keratinized epithelial cells lacking nuclei. Finally, during the metestrus stage, there is a coexistence of nucleated epithelial cells, leukocytes, and keratinized epithelial cells lacking nuclei. The red arrows indicate epithelial cells, the black arrows indicate leukocytes, and the blue arrows indicate keratinized epithelial cells lacking nuclei. Scale bar = 100 μm.



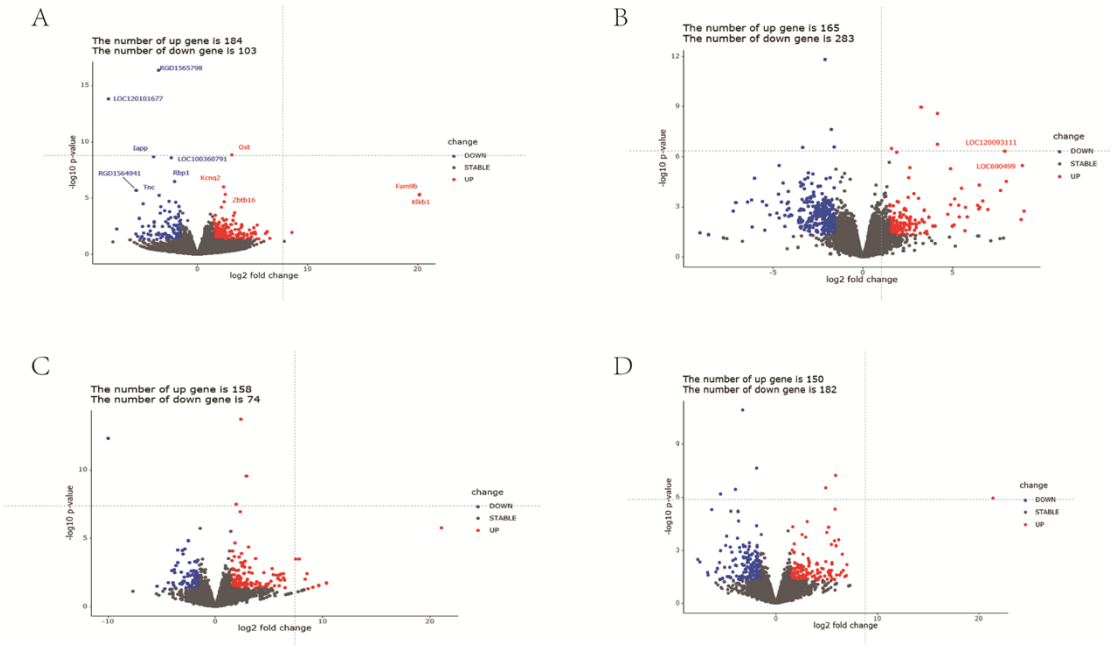
Supplementary Figure 2. The development of model rats with endometrium injury by the surgery to open its abdominal cavity in anesthetized rats.

Small incision was made near the vaginal opening on the right side of the rat uterus, and a curette was used to vertically scrape the endometrium. Subsequently, rat uterus and abdominal cavity were sutured sequentially.



Supplementary Figure 3. Volcano plot of gene expression in each group compared with Con group.

(A) Volcano plot of gene expression in Model group and Con group. **(B)** Volcano plot of gene expression in Gel group and Con group. **(C)** Volcano plot of gene expression in MenSC/Gel group and Con group. **(D)** Volcano plot of gene expression in $MenSC_{SP}$/Gel group and Con group.



Supplementary Figure 4. Volcano plot of gene expression in each group compared with Model group.

(A) Volcano plot of gene expression in Con group and Model group. **(B)** Volcano plot of gene expression in Gel group and Model group. **(C)** Volcano plot of gene expression in MenSC/Gel group and Model group. **(D)** Volcano plot of gene expression in $MenSC_{SP}$/Gel group and Model group.

Supplementary Table 1. Antibodies and reagent kit used in this study

Name	Catalog Number	Company
CD44	203906	Biolegend
CD90	206105	Biolegend
CD105	323214	Biolegend
CD34	343502	Biolegend
CD45	304002	Biolegend
CCK8	CK04	DOJINDO
CellTiter-Glo	G9681	Promega
AnnexinV-PI Apoptosis Kit	C1062L	Beyotime
TUNEL Kit	C1086	Beyotime
DAPI	C1006	Beyotime
CD31	NB100-64796SS	Novus
Alexa 488	4408S	Cell Signaling Technology

Supplementary Table 2. The table presenting all hydrogel concentration gradients

Gelatin concentration (w/v)	mTG Enzyme activity unit
5%	10 U/g
5%	20 U/g
5%	30 U/g
10%	10 U/g
10%	20 U/g
10%	30 U/g
20%	10 U/g
20%	20 U/g
20%	30 U/g

Supplementary Table 3. Primers used in this study

Name	Primer	sequence
GAPDH	F-Primer	CAGTGCCAGCCTCGTCTCAT
GAPDH	R-Primer	AGGGGCCATCCACAGTCTTC
ORM1	F-Primer	GACTGGACAAAGGATAAGTGCAG
ORM1	R-Primer	GGATCCTTCTTGGTCTCCTTC
H19	F-Primer	GATGGAGAGGACAGAAGGACAGT
H19	R-Primer	GAGAGCAGCAGAGATGTGTTAGC
HOMX1	F-Primer	GCACTATGTAAAGCGTCTCCACGAG
HOMX1	R-Primer	CCAGGCAAGATTCTCCCTTACAGAG