Science Advances
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Supplementary Materials for

In situ repair abilities of human umbilical cord–derived mesenchymal stem cells and autocrosslinked hyaluronic acid gel complex in rhesus monkeys with intrauterine adhesion

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Published 22 May 2020, *Sci. Adv.* **6**, eaba6357 (2020)
DOI: 10.1126/sciadv.aba6357

This PDF file includes:

Supplementary Materials:

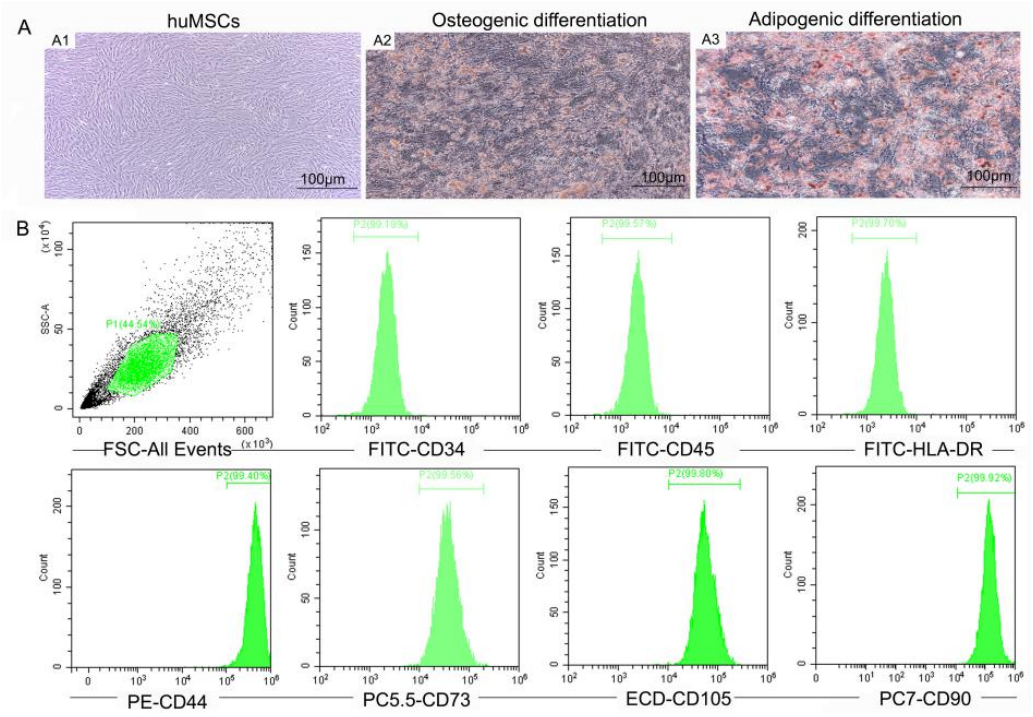

Fig. S1. huMSCs characteristics and differentiation potential. (A) The morphologic and intrinsic characterization of huMSCs. A1: Image of huMSCs derived from umbilical cord at passage 4, which showed the typical fibroblast-like morphology; A2/A3:The most representative differentiation potential image of huMSCs. (B) Surface markers characteristic of huMSCs measured by flow cytometry.

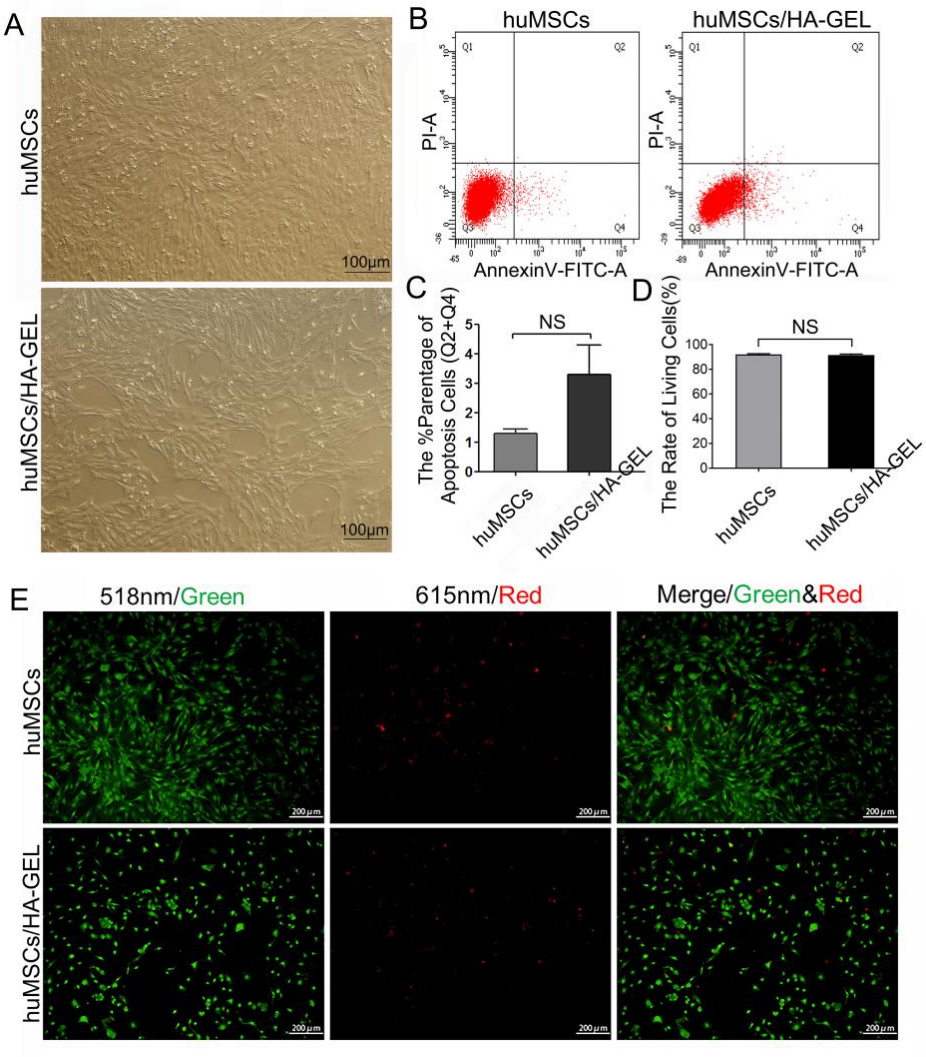

Fig. S2. Safety assessment and verification of huMSCs on an autocrosslinked HA gel. (A) The most representative image of huMSCs in the culture-separated group (huMSCs) and the coculture group (huMSCs/HA-GEL). (B) The most representative images of apoptosis by FACS. (C) The change of apoptosis index (AI) in the two groups. “NS” represents “no significant difference vs. The corresponding culture-separated group”. (D) The rate of living cells in the two group after Live-Dead cells staining. “NS” represent no significant difference. (E) The most representative images of Live-Dead cells in the two

groups. (Green, live cells; Red, Dead cells). the results shown are the mean ± SEM of three replicates from each experiment.

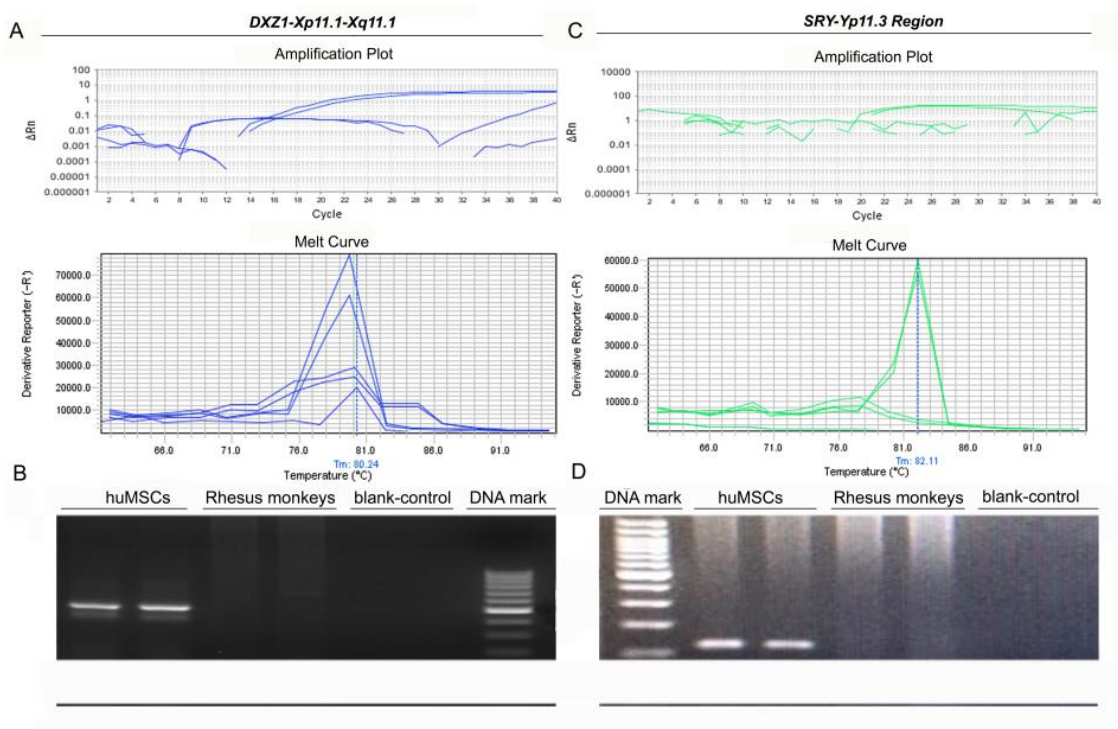

Fig. S3. Homology verification of the two probe sequences for the Yp11.3 region (SRY) and DXZ1 (Xp11.1-Xq11.1) between human and rhesus monkeys. (A) The amplification plot and melt curve of DXZ1. (B) The most representative images of agarose gel electrophoresis for nucleic acid amplification products of DXZ1. (C) The amplification plot and melt curve of SRY. (D) The most representative images of agarose gel electrophoresis for nucleic acid amplification products of SRY.

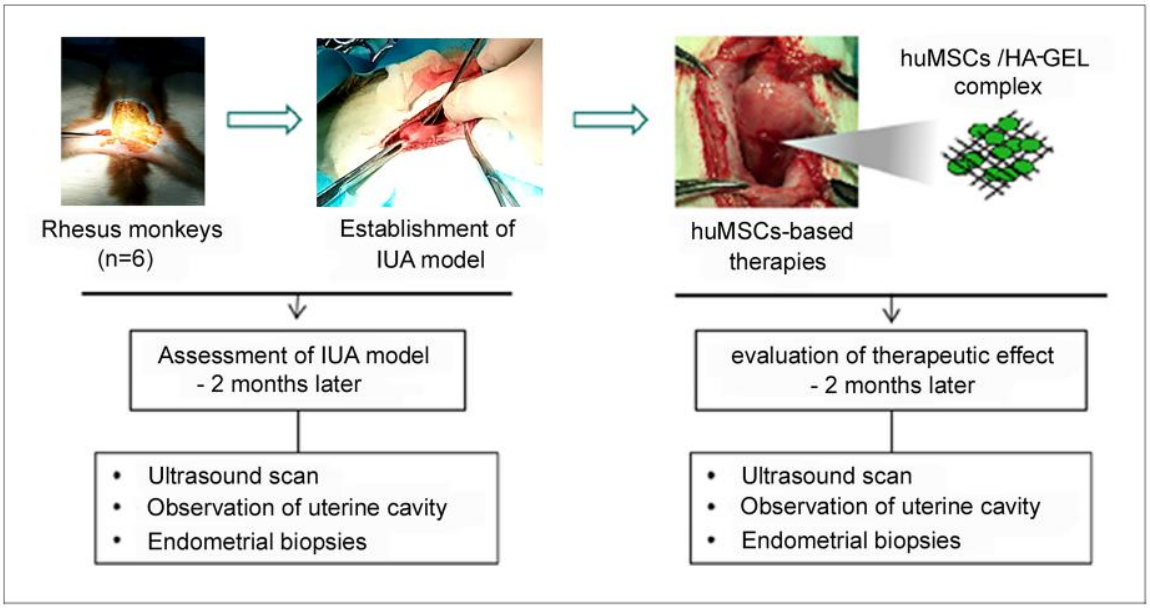

Fig. S4. A flow chart of the whole experimental process. Photos provided by Lingjuan Wang, Chengliang Xiong (Institute of Reproductive Health, Center of Reproductive Medicine, Tongji Medical College, Huazhong University of Science and Technology).

Table S1.

Intrauterine changes and histological inspection of the pre- and post- dilatation and curettage (D&C).

Table S1. Intrauterine changes and histological inspection of the pre- and post- dilatation and curettage (D&C).

Variable	Pre-D&C	2 months Post-D&C
Endometrial thickness (mm)	4.0333±0.5185	1.9833±0.4298**
Number of glands (/mm^2)	6.8576±2.6901	0.6839±0.8608***
Fibrotic areas ratio (%)	0.0716±0.0942	0.6557±0.6359*
Weight of rhesus monkeys (kg)	5.2417±0.3983	5.2333±0.4643

D&C: represent the acronym of “dilatation and curettage”.

*P<0.05,**P<0.01,***P<0.001, all vs. Pre-D&C

Table S2.

The effect of huMSCs/HA-GEL or HA-GEL on intrauterine changes and histological inspection.

Table S2. The effect of huMSCs/HA-GEL or HA-GEL on intrauterine changes and histological inspection

Variable	2 months Post-HA-GEL	2 months Post-huMSCs/HA-GEL
Endometrial thickness (mm)	1.0667±0.6650	4.2667±0.5558$^{\#\#}$
Number of glands (/mm^2)	3.6320±1.0060	4.9662±1.4935$^{\#\#}$
Fibrotic areas ratio (%)	14.2131±13.7193	5.5955±3.6572$^{\#\#}$
Weight of rhesus monkeys (kg)	4.9000±0.2160	5.7333±0.3424$^{\#}$

$^{\#}P<0.05$,$^{\#\#}P<0.01$, all vs. 2 months Post-HA GEL.

Table S3.

The therapeutic effect of HA-GEL or huMSCs/HA-GEL before and after transplantation.

Table S3. The therapeutic effect of HA-GEL or huMSCs/HA-GEL before and after transplantation in situ

Variable	HA-GEL			huMSCs/HA-GEL		
	Pre-D&C	2 months Post-D&C	2 months Post-HA-GEL	Pre-D&C	2 months Post-D&C	2 months Post-huMSCs/HA-GEL
Endometrial thickness (mm)	4.03±0.52	1.98±0.41 **	1.07±0.67 $^{\#'\#'\#'}$	4.03±0.52	1.98±0.41 **	4.27±0.56 $^{\#\#}$ $^{\#''\#''}$
Number of glands (/mm^2)	6.86±2.69	0.68±0.86 ***	3.63±1.01 $^{\#'\#'}$ $^{\#''\#''\#''}$	6.86±2.69	0.68±0.86 ***	4.97±1.49 ^{ns} $^{\#''\#''\#''}$
Fibrotic areas ratio (%)	0.07±0.09	0.66±0.64 *	14.21±13.72 $^{\#'}$ $^{\#''}$	0.07±0.09	0.66±0.64 *	5.60±3.66 $^{\#'\#'}$ $^{\#''\#''}$
Weight of rhesus monkeys (kg)	5.24±0.40	5.23±0.46	4.90±0.22	5.24±0.40	5.23±0.46	5.73±0.344 $^{\#}$

D&C: represent the acronym of “dilatation and curettage”. *P<0.05,**P<0.01,***P<0.001, all vs. Pre-D&C; $^{\#'\#'\#'}$P<0.001, vs. Pre-D&C; $^{\#''}$P<0.05,$^{\#''\#''}$P<0.01,$^{\#''\#''\#''}$P<0.001, all vs. 2 months Post-D&C. ns: no significant difference vs. Pre-D&C

Table S4.

The basic information of rhesus monkeys.

Table S4. The basic information of rhesus monkeys.

Rhesus monkeys	Lot. No.	Age (years)	Gender	Weight (kg)
10401	815	6	Female	5.25
10402	801	6	Female	5.60
10403	607	7	Female	4.65
10404	802	6	Female	5.10
10405	803	6	Female	5.45
10406	804	6	Female	5.25

Lot. No.: represent the lot number of monkeys.

Table S5.

Primers of each target gene used in qRT-PCR analyses.

Table S5. Primers of each target gene used in qRT-PCR analyses.

Gene		Sequence (5′-3′)	Nucleotide base
DXZ1	Forward primer	ATAATTTCCCATAACTAAACACA	23
	Reverse primer	TGTGAAGATAAAGGAAAAGGCTT	23
SRY	Forward primer	AGAGAATCCCAGAATGCGAAAC	22
	Reverse primer	CTTCCGACGAGGTCGATACTT	21
IFN-γ	Forward primer	AATGTCCAACGCAAAGCAGT	20
	Reverse primer	TCACTGGGATGCTCTTCGAC	20
IL-4	Forward primer	CTCTGTGCACCAAGTTGACC	20
	Reverse primer	GTCGAGCCGTTTCAGGAATC	20
IGF-1	Forward primer	AGGTTGGCCAAAGACACATC	20
	Reverse primer	TGCTGTCTGCTCCTCTCTCA	20
EGF	Forward primer	CCAGTGCTCAGAAGGCTACC	20
	Reverse primer	TGTGCAGTTGGCATTCTCTC	20
GAPDH	Forward primer	AGGTCGGAGTCAACGGATTT	20
	Reverse primer	CATTGATGACGAGCTTCCCG	20