Supplemental information

Supplemental file 1. Detailed methods of isolation, characterization, shotgun proteomic and bioinformatics analysis of MenSCs-sEVs

Supplemental file 2.mp4 3D immunofluorescence video of rat uteri labeled with CK-18 after treated with MenSCs-sEVs (green: CK-18, red: MenSCs-sEVs)

Supplemental file 1

Detailed methods of isolation, characterization, shotgun proteomic and bioinformatics analysis of MenSCs-sEVs

Isolation of MenSCs-sEVs

After reaching 80% confluency, P3-P6 MenSCs were washed with PBS and cultured in DMEM/F12 without FBS for 24 h. The cell culture supernatant was collected and centrifuged at 2000 ×g for 20 min then filtrated by 0.22 μm filter (Millipore, Billerica, USA). The filtrate was ultracentrifuged at 10,000 g for 1 h and 100,000 g for 4 h at 4 °C (HITACHI CS120FNX, JAPAN). The enriched exosomes were suspended in PBS then stored at −80 °C for further assays and transplantation. Bicinchoninic acid (BCA) protein quantification kit (Beyotime, China, Cat#P0010S) was used to evaluated the protein content of exosomes.

Characterization and labeling of MenSCs-sEVs

Transmission electron microscopy was used to characterize the morphology of MenSCs-sEVs (HITACHI H-7650, JAPAN). Nanoparticle tracking analysis (NTA) was used to characterize the size distribution and concentration of EVs (Zetaview, Particle Metrix, Germany). Western blotting was used to analyzed the expression of EV specific markers CD63 (1:500, Santa Cruz Biotechnology, USA, Cat#sc-365604) and CD81(1:500, Santa Cruz Biotechnology, USA, Cat#sc-7637). EVs were labeled with CM-DiI (Invitrogen, USA, Cat#C70001) to confirm the distribution within the uterus of IUA rats.

Shotgun proteomic and bioinformatics analysis

MenSCs-sEVs were lysed with SDT buffer (2% SDS, 100 mM DTT, 100 mM Tris-HCL) and boiled for 5 min. Samples were separated on 12.5% SDS-PAGE and then stained with Coomassie brilliant blue.

MenSCs-sEVs were again lysed with SDT buffer and boiled for 15 min for mass spectrometry. Protein suspensions were digested at 37 °C overnight. The resulting peptides were separated by chromatography and analyzed by a Q-Exactive mass spectrometer (Thermo Scientific). Finally, the data were investigated with Mascot server version 2.2 (Matrix Science, UK) using the human UniProt protein database.

Functional annotation of identified proteins was employed using the (http://wego.genomics.org.cn/). The enriched pathways were visualized using the Cytoscape (v3.5.0) ClueGO (v.2.5.0), associated with the Kyoto Encyclopaedia of Genes and Genome (KEGG) database [15]. Briefly, a kappa score of 0.4 and medium specificity were used to explored KEGG pathways. A two-sided hypergeometric test was used as the enrichment method and p-value < 0.05 were considered significant.