Supplementary Methods

Isolation and characterization of SMSCs

Isolation of SMSCs

Synovial membrane tissue was aseptically harvested from the knee joint of trauma patients, aged from 30 to 35 years old, during arthroscopically-assisted surgery. Synovium was minced, digested with 0.2% collagenase type I (Gibco brand, Thermo Fisher Scientific, Waltham, MA, USA) in high-glucose Dulbecco's modified Eagle's medium (high-glucose DMEM; Thermo Fisher Scientific) supplemented with 10% foetal bovine serum (FBS; Gibco) and incubated at 37°C overnight. After centrifuging at 300 × *g* for 5 min, cells were collected, washed twice with phosphate-buffered saline (PBS) supplemented with penicillin-streptomycin (PS; 100 units/mL penicillin, 100 μg/mL streptomycin, Gibco), resuspended in complete culture medium—high-glucose DMEM supplemented with 10% FBS and PS—then plated into T25 culture flasks and incubated at 37°C with 5% humidified CO_2. After incubating for 4 days to allow cell attachment, the complete culture medium was refreshed every three days. After 14 days, cells were digested using trypsin-EDTA (0.25%; Gibco) and counted. Some of the primary cultured cells were preserved at -80°C using CELLSAVING (New Cell & Molecular Biotech, Suzhou, China). The remainder were cultured and passaged for follow-up study.

Multiple differentiation potential

At passage five, the cells were induced to differentiate by switching to osteogenic,

adipogenic or chondrogenic differentiation medium. Osteogenic differentiation was induced by replacing the complete culture medium with osteogenic medium (Cyagen, Suzhou, China) and culturing for 2 weeks. For adipogenic differentiation, the StemPro® Adipogenesis Differentiation Kit (Gibco) was used for 2 weeks. For chondrogenic induction, SMSCs were encapsulated in alginate gel beads and cultured using a StemPro® Chondrogenesis Differentiation Kit (Gibco) for 4 weeks. The procedure of inducing differentiation was as described in the instruction handbook.

Surface markers of SMSCs

Flow cytometry was used to analyse the surface antigens of SMSCs. At passage five, cells were harvested using trypsin-EDTA and incubated with 3% bovine serum albumin (BSA; Gibco) in PBS for 1 h to block non-specific antigen binding. After blocking, the cells were then incubated with the following antibodies (all from Becton, Dickinson and Company; BD): allophycocyanin (APC)-conjugated anti-CD34, phycoerythrin (PE)-conjugated anti-CD44, fluorescein isothiocyanate (FITC)-conjugated anti-CD45, PE-conjugated anti-CD73, APC-conjugated anti-CD90, FITC-conjugated anti-CD105, PE-conjugated anti-CD133 and PE-conjugated anti-CD151. The stained cells were analysed using a Guava® easyCyte™ flow cytometer (Merck–Millipore, Darmstadt, Germany).

Isolation and identification of exosomes

Isolation of exosomes

When SMSCs reached 50–60% confluence they were washed with PBS and cultured in MesenGro® hMSC medium (StemRD, Burlingame, CA, USA) for an additional 48 h at 37°C in an atmosphere of 5% humidified CO_2. The conditioned medium (CM) was collected and centrifuged at 300 × *g* for 15 min at 4°C and then at 2,500 × *g* for 15 min to remove dead cells and cellular debris. After centrifugation, the supernatant was filtered using a 0.22 μm filter (Merck–Millipore) to remove the remaining cells and cellular debris. Then the filtered solution was transferred to a 15 mL Amicon Ultra-15 Centrifugal Filter Unit (Merck–Millipore) and centrifuged at 4,000 × *g* until the volume in the upper compartment was concentrated to approximately 200 μL. The ultrafiltered liquid was washed with PBS and centrifugation was repeated three times. The washed ultrafiltration liquid containing exosomes was laid on top of a 30% sucrose/D_2O cushion in a sterile Ultra-Clear™ tube (Beckman Coulter, Brea, CA, USA) and ultracentrifuged at 100,000 × *g* for one hour at 4°C. The pellets were resuspended in 15 mL PBS and centrifuged at 4,000 × *g* to concentrate the volume to approximately 200 μL. Measurement of the exosome particle number was performed using a CD63 ExoELISA™ kit (System Biosciences, Palo Alto, CA, USA) following the manufacturers' instructions.

Dynamic light scattering (DLS) analysis of exosomes

The size distribution of the exosomes was measured using Nanosizer™ technology (Malvern Instruments, Malvern, UK), and was analysed using Zetasizer software (Malvern).

Transmission electron microscopy (TEM) analysis of exosomes

The morphology of the exosomes was observed by transmission electron microscopy (TEM). The exosomes were loaded onto copper grids coated with Formvar (Structure Probe, Inc., PA, USA). The grids were contrasted using 2% uranyl acetate, dried and then observed using a Philips Morgagni 268D microscope (Philips, Amsterdam, Netherlands).

Isolation of RNA and protein from exosomes

RNA and protein were extracted from exosomes using a Total Exosome RNA & Protein Isolation Kit (Invitrogen, Carlsbad, CA, USA) following the instructions supplied. The obtained RNA and protein were subsequently used in qPCR and western blotting to detect the components of the exosomes.

Exosome uptake by chondrocytes

SMSCs were labelled with the fluorescent lipophilic tracer Vybrant DiO (green) dye (Molecular Probes, Carlsbad, CA, USA) following the manufacturers' instructions. Briefly, the SMSCs were digested using trypsin-EDTA before being resuspended in 1 mL MesenGro® hMSC medium. After adding 5 μL of DiO solution into the suspension, the mix was incubated at 37°C in 5% humidified CO_2 for 15 min. After the mix was centrifuged at 300 × *g* for 10 min and washed three times using PBS, cells were cultured in complete medium until they reached 50–60% confluence.

Exosomes were isolated using the protocol described above and incubated with chondrocytes for 6 h. After washing three times in PBS to remove free exosomes, the chondrocytes were fixed with 4% paraformaldehyde for 15 min and stained with DAPI for 5 min. Finally, the images were photographed using a Leica DMI6000B fluorescence microscope (Leica Microsystems, Wetzlar, Germany).

Real-time quantitative polymerase chain reaction (qPCR)

The sequences of miRNA are shown below:

reverse transcription primer for RNU6B:

5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAAAAATAT-3'

forward primer for RNU6B:

5'-CAAGGATGACACGCAAAT-3'

reverse primer for RNU6B:

5'-TGGTGTCGTGGAGTCG-3'

reverse transcription primer for miR-140-5p

5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCTACCATA-3'

forward primer for miR-140-5p

5'-AGTGGTTTTACCCTATGG-3'

reverse primer for miR-140-5p

5'-AACTGGTGTCGTGGAG-3'

The primer sequences of mRNA are shown below:

WNT1-FOR: 5’-GTCCTCCTAAGTCCCTTCCT-3’

WNT1-REV: 5’-CCTTTTGCCCTGTAACCTC-3’

WNT2-FOR: 5’-TGGGGATACAAGATTGGTGAA-3’

WNT2-REV: 5’-ATGGGCATTAGCAGACGG-3’

WNT2B-FOR: 5’-GATGGGACAAAGATGAATGG-3’

WNT2B-REV: 5’-GGCTCTCAATAATAGAATGT-3’

WNT3-FOR: 5’-GGATTCAGCGAAGTCTCAT-3’

WNT3-REV: 5’-AGTTGGGTCTGGGTCATTTAC-3’

WNT3A-FOR: 5’-GCCATCCTCTGCCTCAAAT-3’

WNT3A-REV: 5’-CGTCTAACTCCGTTGGACAG-3’

WNT4-FOR: 5’-CCACGCAACGCACAGTTCAAG-3’

WNT4-REV: 5’-AAGTCGGGGCTAGGCTCCAAGT-3’

WNT5A-FOR: 5’-CTTGAGCACGACGAAGCAAC-3’

WNT5A-REV: 5’-CAACCCAACACGCATTTCAG-3’

WNT5B-FOR: 5’-TCTTCATCCTCCACGGTT-3’

WNT5B-REV: 5’-CAGTTTAGGGCTTTCCTGAC-3’

WNT6-FOR: 5’-AGAGTGCCAGTTCCAGTTCC-3’

WNT6-REV: 5’-GTCTCCCGAATGTCCTGTTG-3’

WNT7A-FOR: 5’-GCCATCATCGTCATAGGAGAA-3’

WNT7A-REV: 5’-GCCATTGCGGAACTGAAAC-3’

WNT7B-FOR: 5’-AGGATTCTCGGCACTAACA-3’

WNT7B-REV: 5’-GGAAAGGAAACAGAGGGT-3’

WNT8A-FOR: 5’-AGAACAGCCACAACACATCC-3’

WNT8A-REV: 5’-CAGTTTTCCTCTCTTCCACCT-3’

WNT9A-FOR: 5’-AGTACAGCAGCAAGTTCGT-3’

WNT9A-REV: 5’-GAGGTTGTTGTGGAAGTC-3’

WNT10A-FOR: 5’-CCACGAATGCCAACACCAA-3’

WNT10A-REV: 5’-CGGAAACCTCTGCTGAAGATG-3’

WNT11-FOR: 5’-GACTCGGAACTCGTCTATCTG-3’

WNT11-REV: 5’-AGCCCACCTTCTCATTCTT-3’

WNT16-FOR: 5’-GGAAACACCACGGGCAAAG-3’

WNT16-REV: 5’-AGCGGCAGTCTACTGACATCAA-3’

ANKRD1-FOR: 5’-AGCCCAGATCGAATTCCGTG-3’

ANKRD1-REV: 5’-TGAGCAACTTATCTCGGGCG-3’

CTGF-FOR: 5’-TGTGCACCGCCAAAGATGG-3’

CTGF-REV: 5’-ACGTGCACTGGTACTTGCAG-3’

Cyr61-FOR: 5’-AAGGAGCTGGGATTCGATGC-3’

Cyr61-REV: 5’-CATTCCAAAAACAGGGAGCCG-3’

ACAN-FOR: 5’-GCCAGCACCACCAATGTAAG-3’

ACAN-REV: 5’-TTCAGTAACACCCTCCACGA-3’

Col II-FOR: 5’-TGAAGGTGCTCAAGGTCCTC-3’

Col II-REV: 5’-ATTCCATCTGTTCCAGGGTT-3’

SOX9-FOR: 5’-GCTCTGGAGACTTCTGAACG-3’

SOX9-REV: 5’-GGGTGGTCCTTCTTGTGCT-3’

YAP-FOR: 5’-AACCGTTTCCCAGACTACCT-3’

YAP-REV: 5’-GCTCCTCTCCTTCTATGTTC-3’

RalA-FOR: 5’-AGGGGTCCTAACCACTAACA-3’

RalA-REV: 5’-GGCTTCATAGCACCTCAGTA-3’