

Supplementary Materials for

Human amniotic mesenchymal stem cell-derived small extracellular vesicles repair osteoradionecrosis of the jaw

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Running title: hAMSCs-sEVs repair ORNJ

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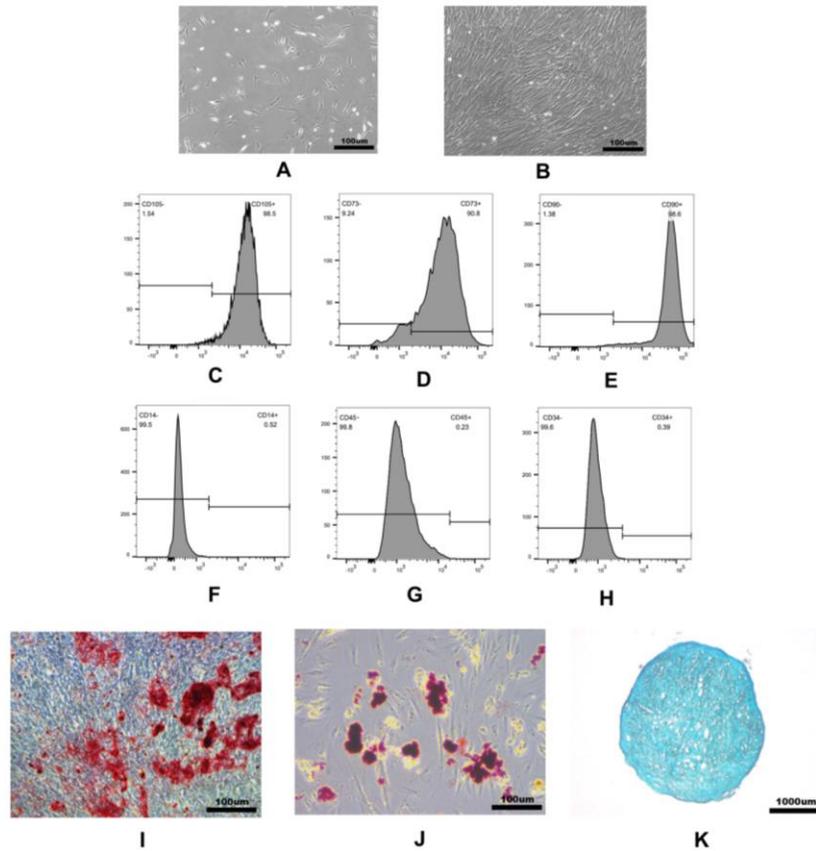
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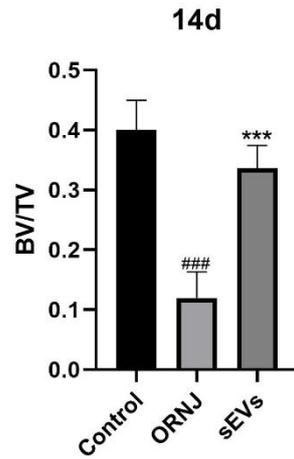
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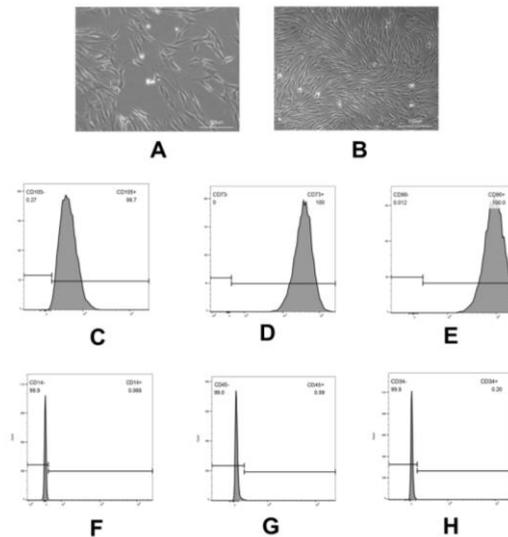
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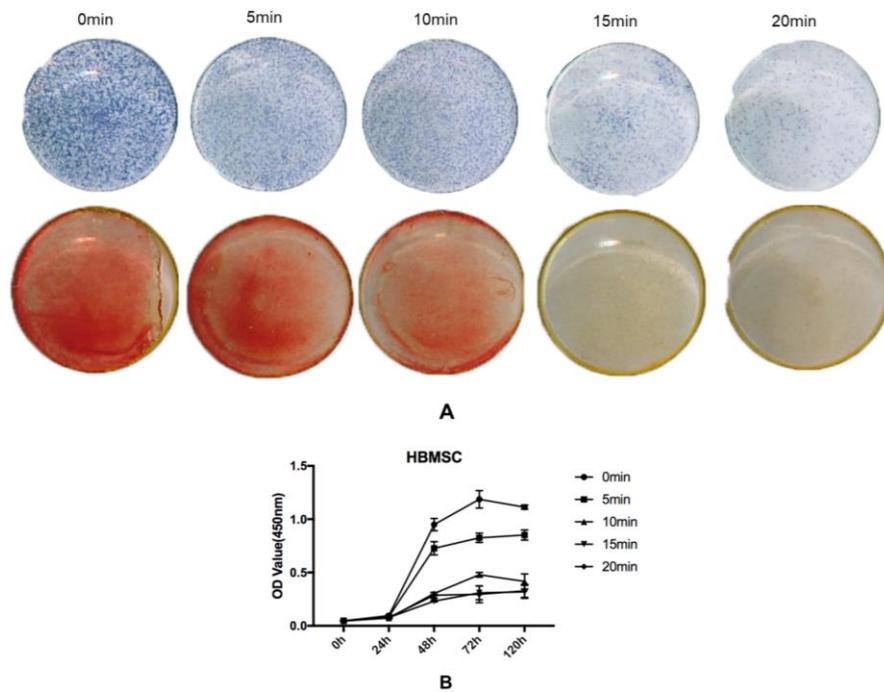
Supplementary Figure 1 *In vitro* culture and identification of hAMSCs. A and B: P0 and P3 culture of hAMSCs (Scale bar = 100µm); C-H: hAMSCs were positive for CD105, CD73, and CD90 but negative for CD14, CD45 and CD34 using fluorescence-activated cell sorting (FACS); I-K: hAMSCs could differentiated to osteoblast (Alizarin red staining, Scale bar = 100µm), adipocyte (Oil Red O staining, Scale bar = 100µm) and chondrocyte (Toluidine blue staining, Scale bar = 1000µm).



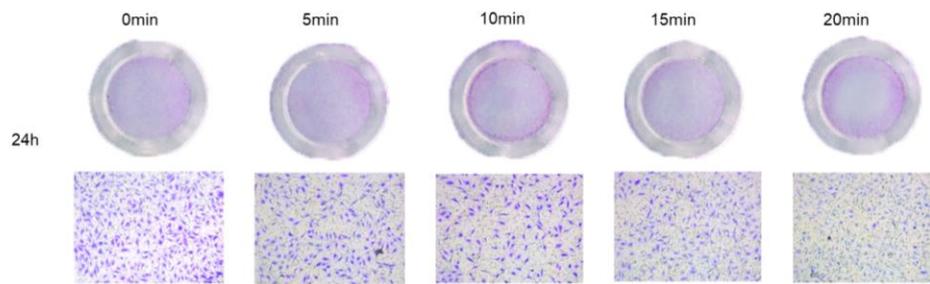
Supplementary Figure 2 BV/TV in Control, 40 Gy and 40 Gy+10¹⁰ hAMSCs-sEVs groups. ### p < 0.001 vs. Control group; *** p < 0.001 vs. 40 Gy group.



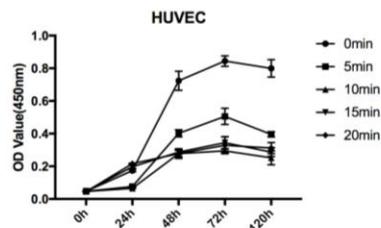
Supplementary Figure 3 *In vitro* culture and identification of BMSCs. A and B: P0 and P3 culture of BMSCs (Scale bar = 100 μ m); C-H: hAMSCs were positive for CD105, CD73, and CD90 but negative for CD14, CD45 and CD34 using fluorescence-activated cell sorting (FACS).



Supplementary Figure 4 Radiation induced inhibition of osteogenesis and proliferation of hBMSCs in a dose-dependent manner. A: Inhibition of osteogenic differentiation by 0,5,10,15 and 20 min exposure of radiation (1.26 Gy/min) using ALP and Alizarin red staining; B: Inhibition of proliferation by 0,5,10,15 and 20-min exposure of radiation (1.26 Gy/min) using CCK-8 assay.



A



B

Supplementary Figure 5 Radiation induced inhibition of migration and proliferation of HUVECs in a dose-dependent manner. A: Inhibition of migration by 0,5,10,15 and 20-min exposure of radiation (1.26Gy/min) using Transwell assay; B: Inhibition of proliferation by 0,5,10,15 and 20-min exposure of radiation (1.26Gy/min) using CCK-8 assay.

Supplementary Table 1 The antibodies used for FACS staining

Antibody	Dilution	Source	Code number
CD73-APC	1:200	EBiocience	17-0739-41
CD105-PE	1:200	EBiocience	12-1057-41
CD90-FITC	1:200	EBiocience	11-0909-41
CD14-PE-Cy7	1:200	EBiocience	25-0149-41
CD45-FITC	1:200	EBiocience	11-0451-82
CD34-APC	1:200	EBiocience	17-0349-41

Supplementary Table 2 The antibodies used for IHC staining

Antibody	Dilution	Source	Code number
COL-1	1:200	Proteintech	26155-1-AP
ALP	1:200	Proteintech	16001-1-AP
CD31	1:200	Proteintech	60269-1-IG
VEGF	1:200	Proteintech	26155-1-AP

Supplementary Table 3 The antibodies used for western blotting

Antibody	Dilution	Source	Code number
TSG101	1:1000	Bioworld	MB11730
CD63	1:1000	Proteintech	25682-1-AP
COL-1	1:1000	Proteintech	26155-1-AP
ALP	1:1000	Proteintech	16001-1-AP
RUNX2	1:1000	Proteintech	22226-1-AP
OPN	1:1000	Proteintech	10084-2-AP
p-AKT	1:1000	Proteintech	66444-1-Ig
AKT	1:1000	Proteintech	10176-2-AP
VEGF	1:1000	Proteintech	66828-1-Ig
CD31	1:1000	Proteintech	33075-1-AP
β -catenin	1:1000	Proteintech	66379-1-Ig
β -actin	1:5000	Bioworld	BS6007MH
GAPDH	1:10000	Proteintech	HRP-60004

Supplementary Table 4 The primer sequences used for qRT-PCR

Gene	Forward or Reverse	Sequences (5'-3')
COL-1	Forward	GAGGGCCAAGACGAAGACATC
	Reverse	GAGGGCCAAGACGAAGACATC
ALP	Forward	GTGAACCGCAACTGGTACTC
	Reverse	GAGCTGCGTAGCGATGTCC
RUNX2	Forward	TGGTACTGTCATGGCGGGTA
	Reverse	TCTCAGATCGTTGAACCTTGCTA
OPN	Forward	CTCCATTGACTCGAACGACTC
	Reverse	CAGGTCTGCGAACTTCTTAGAT
β -catenin	Forward	GCTTTCAGTTGAGCTGACCA
	Reverse	AAGTCCAAGATCAGCAGTCTCA
VEGF	Forward	AGGGCAGAATCATCACGAAGT
	Reverse	AGGGTCTCGATTGGATGGCA
β -actin	Forward	CATGTACGTTGCTATCCAGGC
	Reverse	CTCCTTAATGTCACGCACGAT
GAPDH	Forward	GGAGCGAGATCCCTCCAAAAT
	Reverse	GGCTGTTGTCATACTTCTCATGG