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Supplementary Materials for

Stem cell-homing biomimetic hydrogel promotes the repair of osteoporotic bone defects through osteogenic and angiogenic coupling

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Supplementary Information



Fig. S1. Identification of BMSCs from different groups by flow cytometry.

(A) Flow cytometry showed BMSCs from osteoporotic rats were positive for CD44 (100%), CD29 (100%), CD73 (100%), and CD90 (99.9%), negative for CD34 (0.36%) and CD45 (0.35%). (B) Flow cytometry showed BMSCs from osteoporotic rats were positive for CD44 (100%), CD29 (100%), CD73 (99.6%), and CD90 (98%), negative for CD34 (0.49%) and CD45 (1.75%). n = 3 for each group.



Fig. S2. Analyzing the impact of cell membrane coverage on MSNs.

(A) Flow cytometric analysis of MSNs (coated by BMSCs and HUVECs membranes) uptake by BMSCs. (B) Flow cytometric analysis of MSNs (coated by BMSCs and HUVECs membranes) uptake by HUVECs.



Fig. S3. Pore size distributions of SA, SA-MSNs@CM and SA-MSNs@CM-Stiff. The pore size distribution of SA, SA-MSNs@CM and SA-MSNs@CM-Stiff was measured by low-frequency magnetic resonance.





(A) Erythrocyte haemolysis assay to evaluate the biocompatibility of the hydrogel. (B) Hematoxylin and eosin staining and histological analysis of main organs from hydrogels-treated rats. n = 3 for each group.



Fig. S5. SA-MSNs@CM-Stiff induces BMSCs recruitment and promotes osseointegration *in vivo*.

(A) Relative Emcn fluorescence density (Statistical analysis for Figures 8G). (B) Relative Opn fluorescence density (Statistical analysis for Figures 8H). (C) Relative Runx2 fluorescence density (Statistical analysis for Figures 8I). n = 6 for each group. Error bars denote mean \pm SEM; ns, no significance; *p < 0.05, **p < 0.01, and ***p < 0.001.



Fig. S6. SA-MSNs@CM-Stiff regulates macrophage polarization *in vivo*. Representative immunofluorescence staining of iNOS and CD206 in the femoral condyle of an osteoporotic rat eight weeks after hydrogel implantation (scale bar: $20 \mu m$); n = 6.



Fig. S7. Analytical HPLC trace and electrospray ionization mass spectrometry (ESI-MS) analysis of bone marrow-homing peptide.

(A) Analytical HPLC trace of bone marrow-homing peptide. (B) ESI-MS analysis of bone marrow-homing peptide.

Antibody	Host	Dilution ratio	Company	Item number
anti-Nanog	Rabbit	1:1000	ProteinTech	14295-1-AP
anti-Sox2	Rabbit	1:1000	ProteinTech	11064-1-AP
anti-Oct4	Rabbit	1:1000	ProteinTech	11263-1-AP
anti-P21	Rabbit	1:1000	ProteinTech	10355-1-AP
anti-P53	Rabbit	1:1000	ProteinTech	10442-1-AP
anti-P16	Rabbit	1:600	ProteinTech	10883-1-AP
anti-eNOS	Rabbit	1:1000	ABclonal	A20985
anti-p-eNOS	Rabbit	1:1000	ProteinTech	28939-1-AP
anti-sGC	Rabbit	1:1000	ProteinTech	11936-1-AP
anti-PKG	Rabbit	1:1000	ProteinTech	21646-1-AP
anti-Runx2	Rabbit	1:1000	Abways Technology	CY5864
anti-Opn	Rabbit	1:1000	ProteinTech	25715-1-AP
anti-CD31	Mouse	1:1000	ProteinTech	66065-2-Ig
anti-Emcn	Rabbit	1:1000	Invitrogen	PA5-115178
anti-CD86	Rabbit	1:1000	ProteinTech	13395-1-AP
anti-CD206	Rabbit	1:1000	ProteinTech	18704-1-AP
anti-Gapdh	Rabbit	1:1000	ProteinTech	60004-1-Ig

Table S1. Primary antibodies information for western blotting.

Antibody	Host	Dilution ratio	Company.	Item number
anti-y-H2AX	Rabbit	1:200	Abcam	ab81299
anti-Runx2	Rabbit	1:200	Abways Technology	CY5864
anti-Opn	Rabbit	1:200	ProteinTech	25715-1-AP
anti-CD90	Mouse	1:200	Invitrogen	14-0900-81
anti-CD31	Mouse	1:1000	ProteinTech	66065-2-Ig
anti-Emcn	Rabbit	1:1000	Invitrogen	PA5-115178
anti-CD68	Mouse	1:200	ProteinTech	66231-2-Ig
anti-CD86	Rabbit	1:200	ProteinTech	13395-1-AP
anti-CD206	Rabbit	1:200	ProteinTech	18704-1-AP
anti-iNOS	Mouse	1:50	Invitrogen	MA5-47726

 Table S2. Primary antibodies information for Immunofluorescence.