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Supporting Information

Osteoimmunity-regulating nanosilicate-reinforced hydrogel for enhancing osseointegration

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Table S1. Composition of different groups of hydrogels

Biohydrogel	$CaCl_2$	MMT	SA	HM
SA	2 wt%	/	3 wt%	/
SA/MMT(SM)	2 wt%	1 wt%	3 wt%	/
SA/MMT/HM-low (SMH-l)	2 wt%	1 wt%	3 wt%	4 μg/ml
SA/MMT/HM-middle (SMH-m)	2 wt%	1wt%	3 wt%	8 μg/ml
SA/MMT/HM-High (SMH-h)	2 wt%	1wt%	3 wt%	16 μg/ml

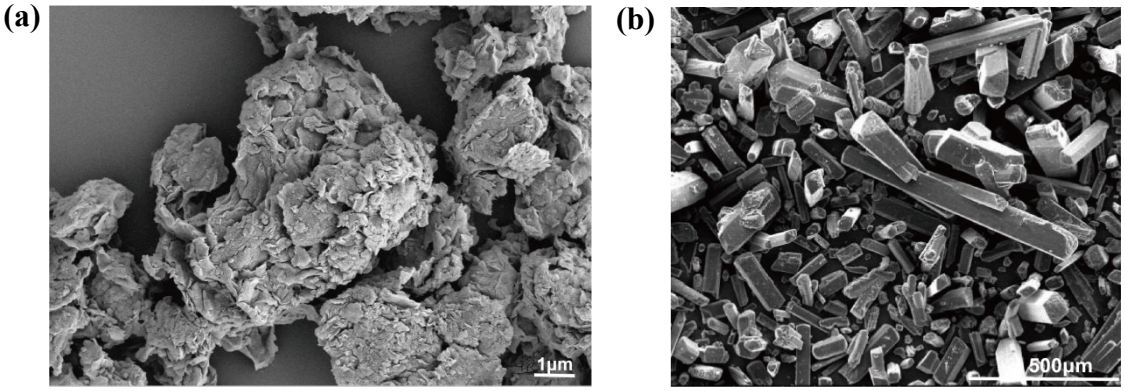

Figure S1. SEM images of (a) MMT (scale bar: 1 μm) and (b) HM (scale bar: 500 μm).

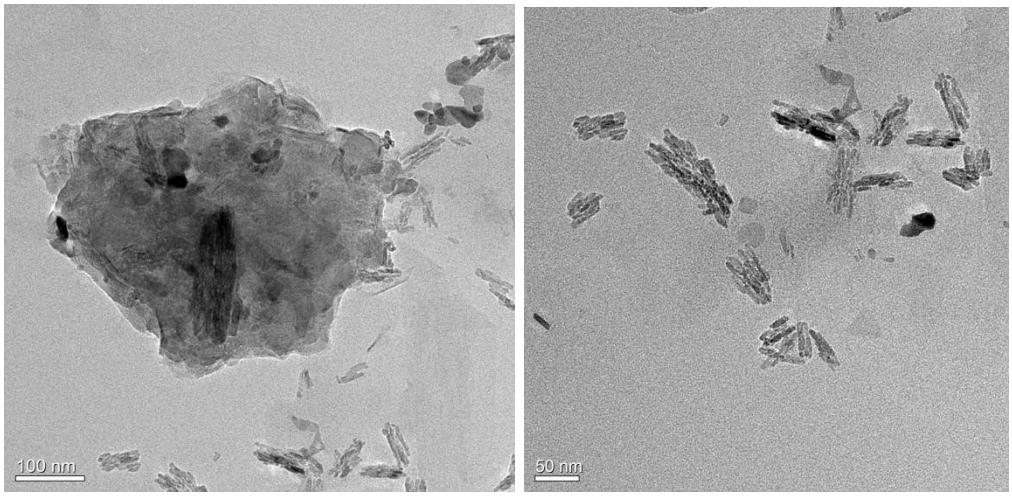

Figure S2. TEM images of MMT (scale bar: 100 nm, left; scale bar: 50 nm, right).

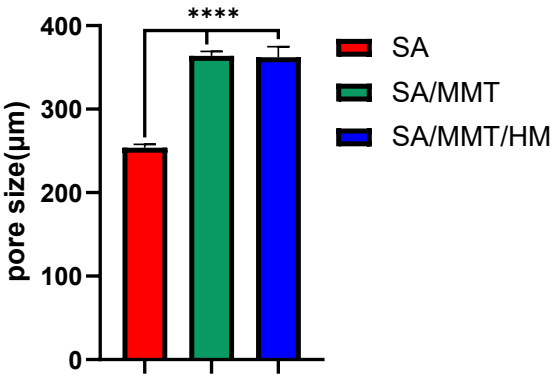

Figure S3. Pore size of SA, SM and SMH hydrogels calculated from the SEM images. ****P < 0.0001

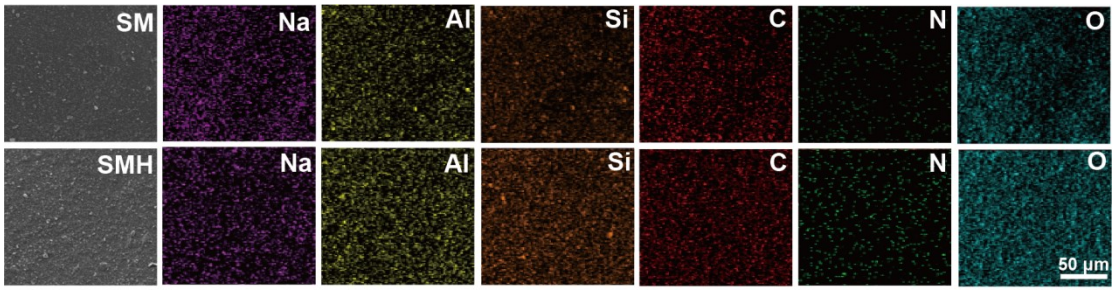

Figure S4. Elemental mapping images of SM and SMH (scale bar: 50 μm).

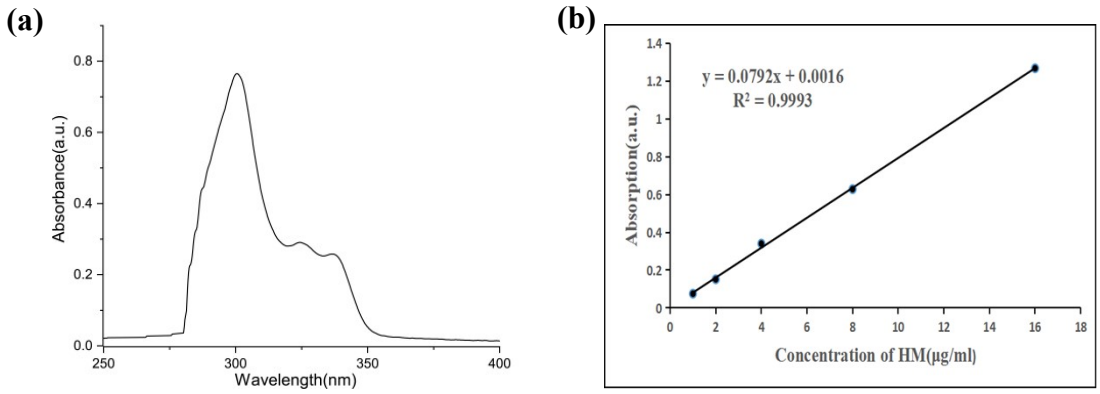

Figure S5. (a) The absorbance spectra of HM. (b) The standard curve of HM detected at 301 nm.

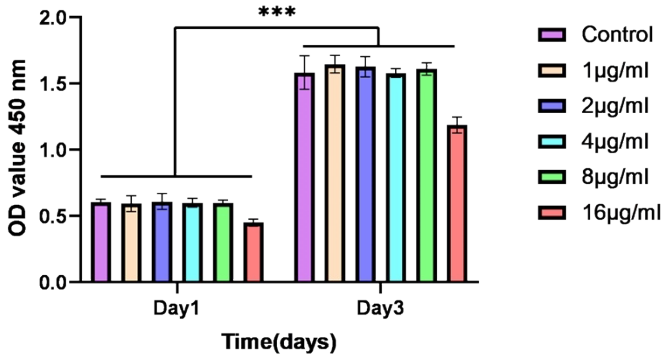

Figure S6. CCK8 assay was used for evaluating cell proliferation following treatment with different concentrations of harmine. ***p < 0.001

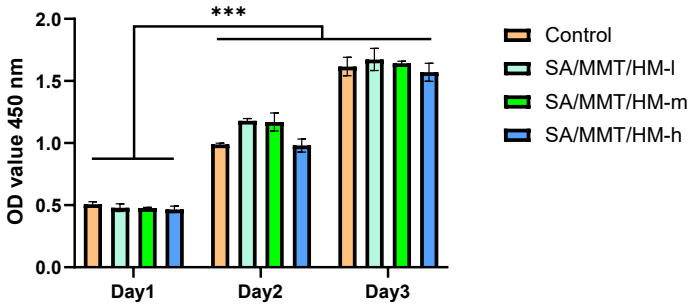

Figure S7. CCK8 assay was used to evaluate the proliferation of RAW264.7 cultured in hydrogel lixivium for 1, 2 and 3 days . ***p < 0.001

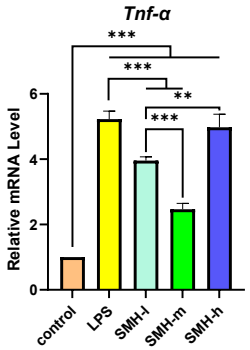

Figure S8. Selecting the optimal concentration group of SMH by inflammatory-related genes test. The mRNA expression levels of TNF-α. *P < 0.05 and **P < 0.01, ***P < 0.001

Table S2. Primers used in the qRT-PCR.

Gene name	5’→3’(Forward)	5’→3’(Reverse)
GAPDH(mouse)	TTCCTACCCCCAATGTATCCG	CATGAGGTCCACCACCCTGTT
TNF-α	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
IL-1β	TGCCACCTTTTGACAGTGATG	AAGGTCCACGGGAAAGACAC
CD86	TGGGCGCAGAGAAACTTGAT	AAGCCCGTGTCCTTGATCTG
IL-6	CAATAGAAGACTGTGAGCATC	CCCATAATCAGCCACCAAACC
IL-10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
PDGF-B	CCTGAGGAACTCTATGAAATGCTGA	GGCGATTACGGCAGGCTCT
CD206	TGCCTACTGCCTGCCCTAATC	GTCCCATCGCTCCACTCAAAG
BMP2	AACGAGAAAAGCGTCAAGCC	AGGTGCCACGATCCAGTCAT
Arg2	ACATTGGCTTGCGAGACGTA	ATCACCTTGCCAATCCCCAG
BMP6	AGCGACACCACAAAGAGTTCA	GCTGATGCTCCTGTAAGACTTGA
ACTB(Rat)	CTCTGTGTGGATTGGTGGCT	CGCAGCTCAGTAACAGTCCG
OCN2	TTATTGTTTGAGGGGCCTGGG	ACACAACTGCAGGTCGAGTTT
OPN	AATGTGCTCCTGGCACCTAC	GCATTCATCACTCGTGTGCC
ALP3	GGCGTCCATGAGCAGAACTACATC	CAGGCACAGTGGTCAAGGTTGG
OSX	GCCTACTTACCCGTCTGACTTTGC	CCCTCCAGTTGCCCACTATTGC
Runx2	CCGTGGCCTTCAAGGTTGTA	ATTTCGTAGCTCGGCAGAGTAGTT

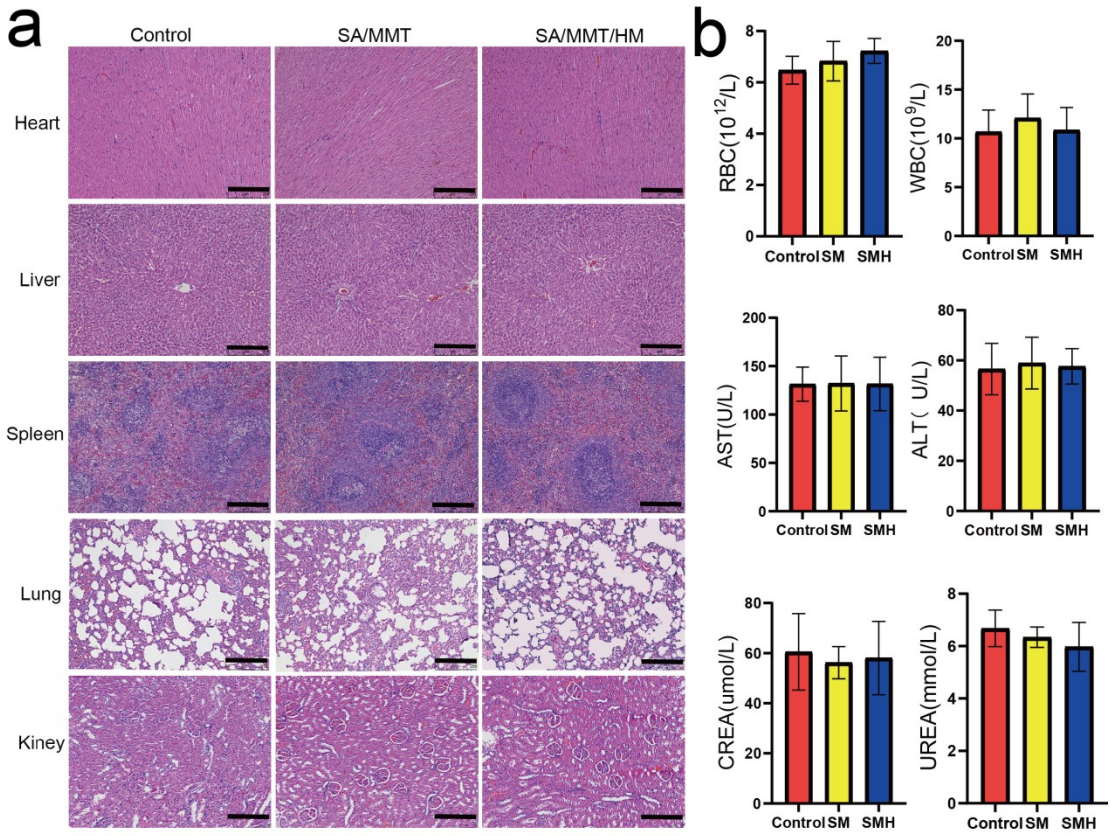

Figure S9. *In vivo* biocompatibility evaluation of SA/MMT/HM. a) H&E staining assessments of rat major organs (heart, liver, spleen, lung, and kidney) after implantation with the hydrogel at the cranial defect site for 4 weeks (scale bar: 200 μm) b) Partial hematological analysis of rat peripheral blood. All statistical data are presented as mean ± SD (n = 3).

Table S3. Antibodies used in immunofluorescence staining of tissues.

Brand and code	Name of primary antibodies	Abbreviation	Host	Secondary antibodies
Abcam，ab125212	Anti-CD68 antibody	CD68	rabbit	Rabbit 647
Sigma-Aldrich，C6198	Anti-Actin, α-Smooth Muscle - Cy3™ antibody, Mouse monoclonal	α-SMA	mouse	/
HUABIO，ET1601-21	Alkaline Phosphatase Recombinant Rabbit Monoclonal Antibody [SA40-00]	ALP	rabbit	Rabbit 647

HUABIO，ER80602	BMP2 Rabbit Polyclonal Antibody	BMP2	rabbit	Rabbit 647
Servicebio，GB11233	Anti -Osteocalcin Rabbit pAb	OCN	rabbit	Rabbit 647

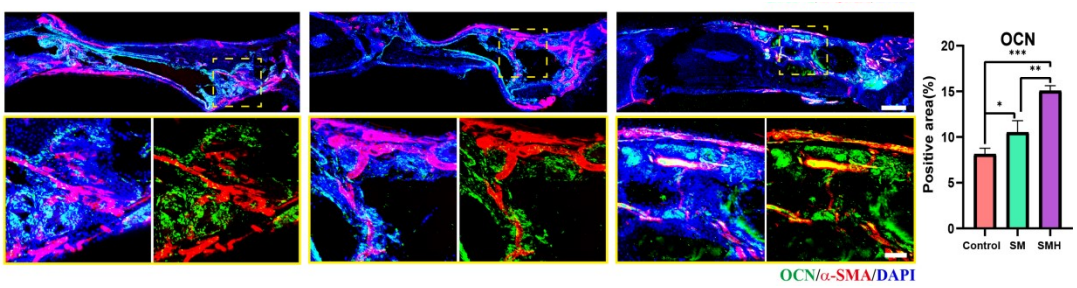

Figure S10. Immunofluorescence staining of cranial defect sections stained with OCN and corresponding quantitative analysis of immunofluorescence staining results. Red (α-SMA), green (OCN), and blue (DAPI). (scale bar: 200 μm, up; scale bar: 50 μm, down).

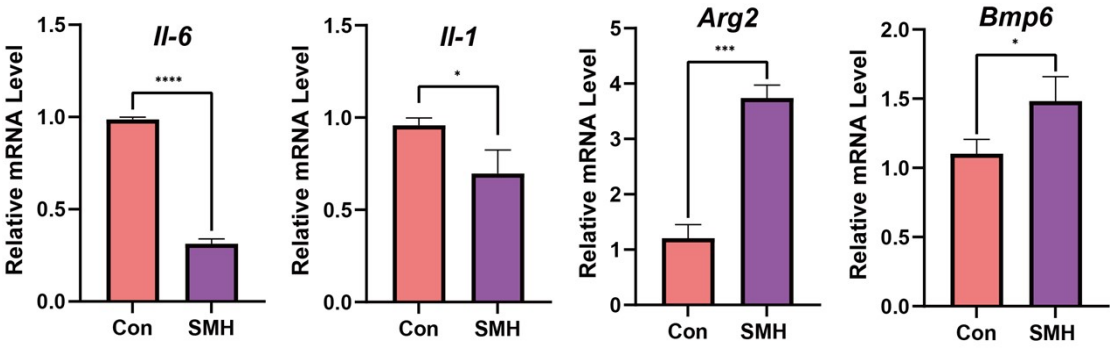

Figure S11. The mRNA expression levels of M1 macrophage–related genes (*Il-6, Il-1*) and M2 macrophage–related genes (*Arg2, Bmp6*). (n=3, *P < 0.05 and **P < 0.01, ***P < 0.001, ****P < 0.0001).