

Bioactive microflower doped triple-network hydrogel for immunomodulation-mediated angio/osteogenesis coordination in mandibular bone regeneration

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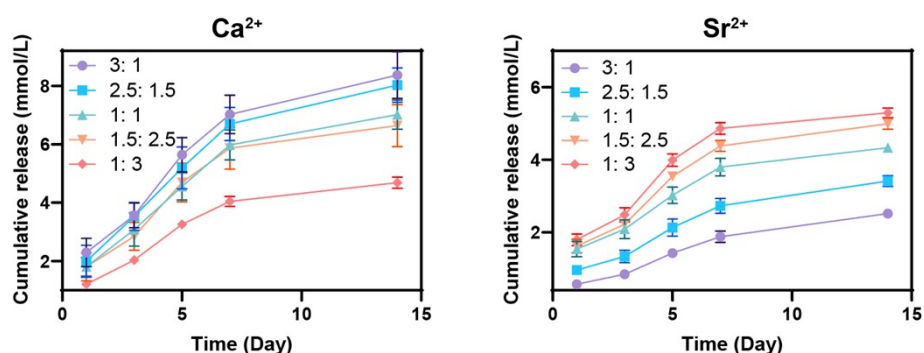


Figure S1. Ion release curves of MFs with different $\text{Ca}^{2+}/\text{Sr}^{2+}$ ratio.

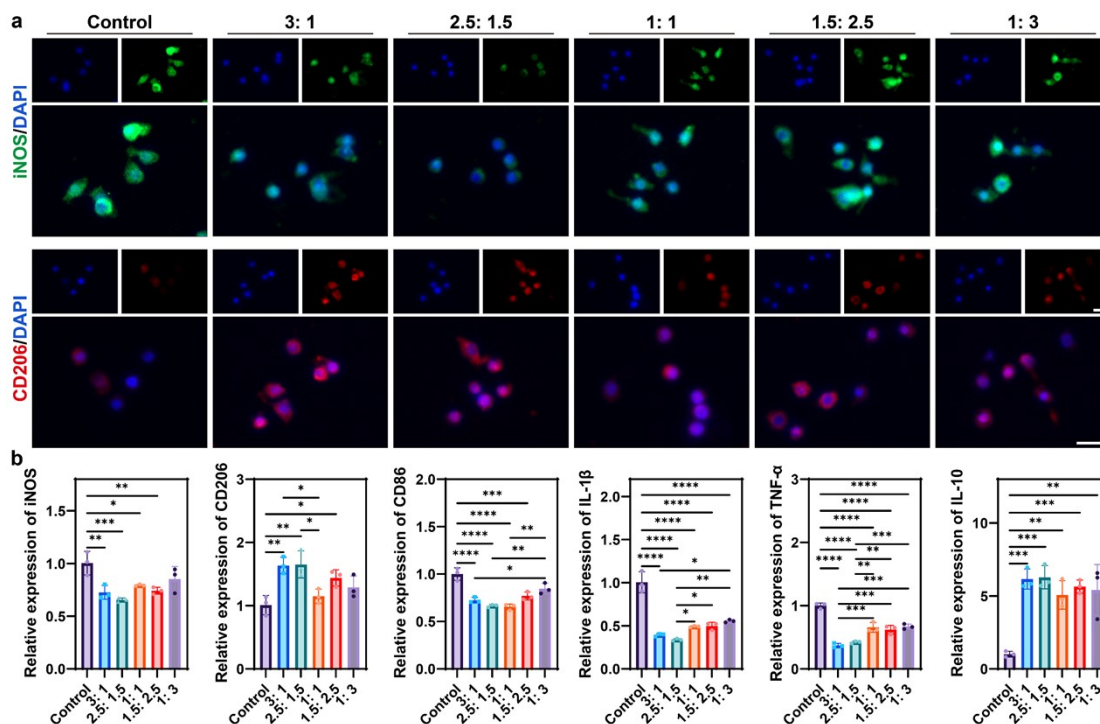


Figure S2. In vitro immunomodulation of macrophages by MFs with different $\text{Ca}^{2+}/\text{Sr}^{2+}$ ratio. a) Immunofluorescence staining of iNOS (green), CD206 (red) and nuclear (blue) in RAW 264.7 cells. Scale bar = 25 μm . b) Relative mRNA expression of iNOS, CD206, CD86, IL-1 β , TNF- α and IL-10 in RAW 264.7 cells. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

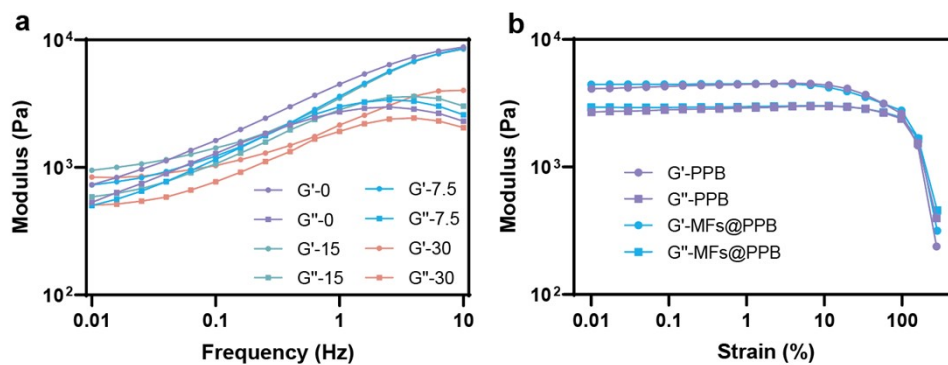


Figure S3. Rheological tests. a) Dynamic frequency sweep test of hydrogels with different MFs concentrations. b) Strain sweep test of PPB and MFs@PPB.

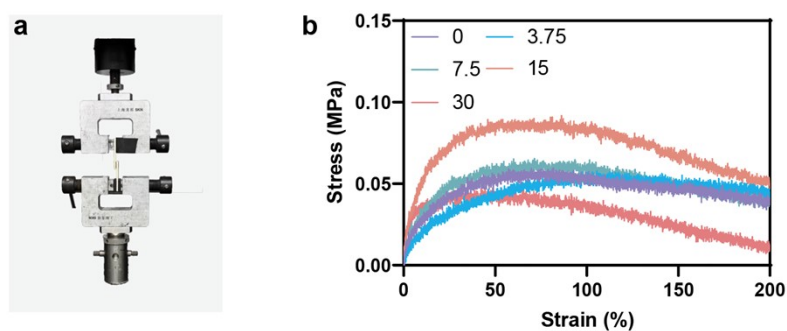


Figure S4. Adhesion test. a, b) Photos and stress-strain curves of hydrogels adhering to bovine bone slice.

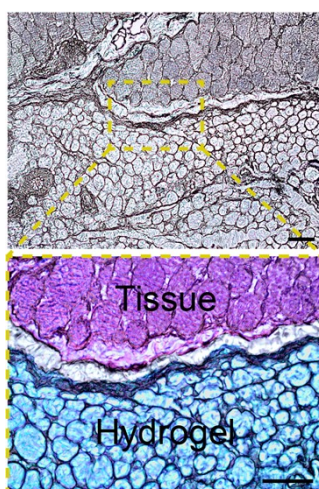


Figure S5. Microscopic images of the adhesion of MFs@PPB to the skin. Scale bar = 100 μ m.

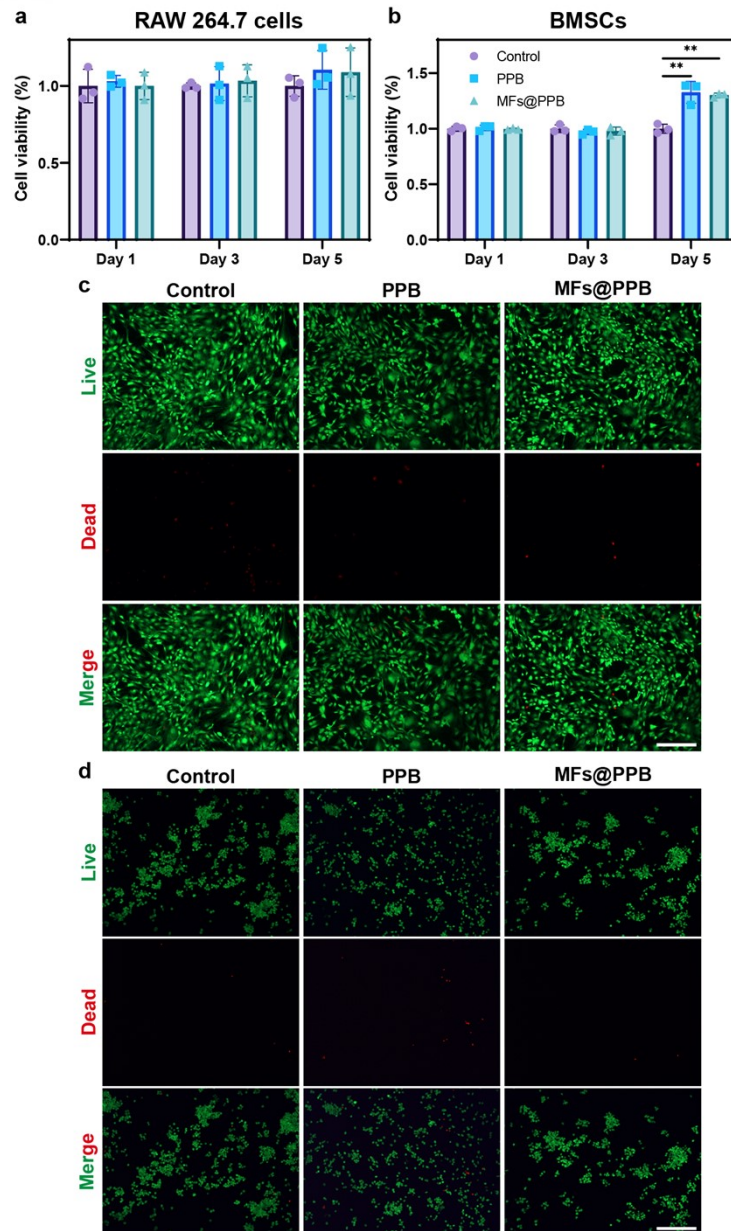


Figure S6. In vitro biocompatibility test. a) CCK-8 tests of RAW 264.7 cells and BMSCs. b) Live/dead staining of BMSCs. Scale bar = 250 μm . c) Live/dead staining of RAW 264.7 cells. Scale bar = 250 μm . (** $p < 0.01$)

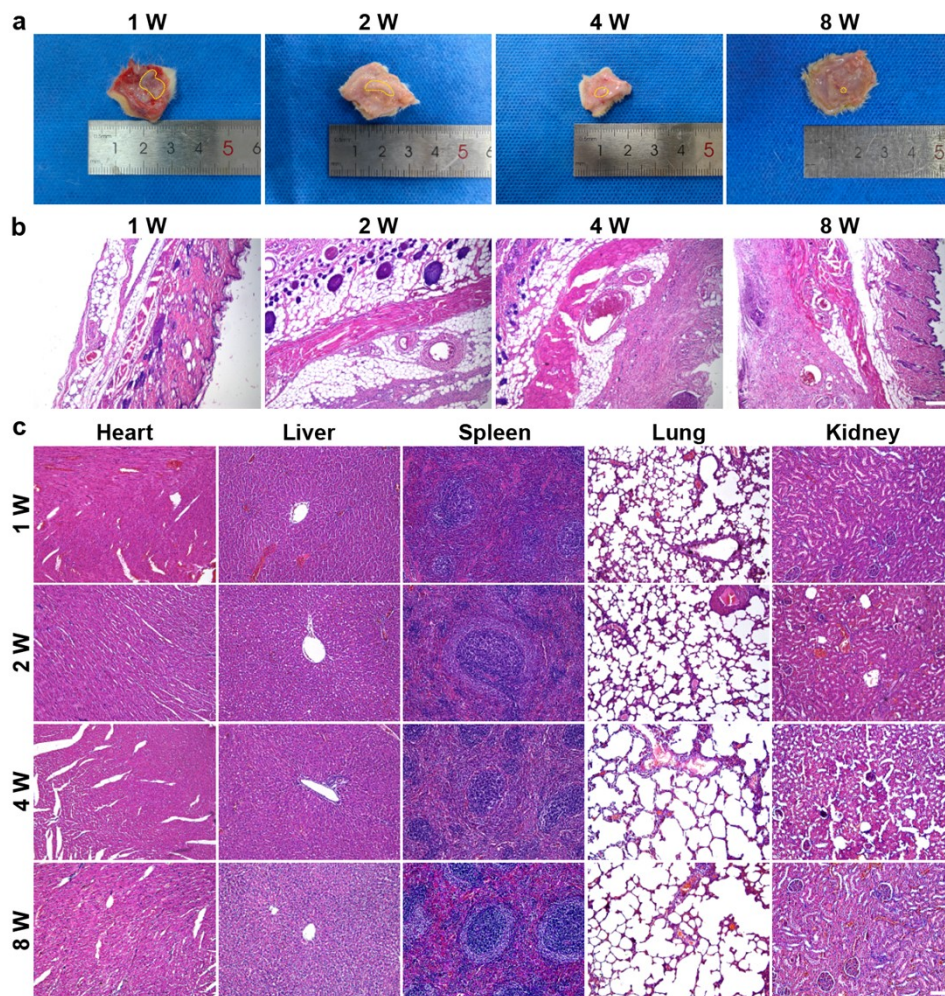


Figure S7. Subcutaneous degradation of MFs@PPB. a) Macroscopic images of the subcutaneous hydrogel at different time points. b) H&E staining of the skins and implanted MFs@PPB hydrogel. Scale bar = 250 μ m. c) HE staining of vital organs, including heart, liver, spleen, lung and kidney. Scale bar = 100 μ m.



Figure S8. Photos of the mouse mandibular bone defect model.

Table S1. Primer sequences used in RT-qPCR analysis.

Gene	Forward primer	Reverse primer
Mouse-GAPDH	AAGGCCGGGGCCACTTGAA	GGACTGTGGTCATGAGCCCTTCC A
TNF- α	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG

IL-1 β	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
CD86	TCAATGGGACTGCATATCTGCC	GCCAAAATACTACCAGCTCACT
iNOS	GTTCTCAGCCCAACAATACAAG A	GTGGACGGGTCGATGTCAC
IL-10	CCCTTTGCTATGGTGCCT	GTGGCCAGTTTGTTATTTAT
CD206	CTCTGTTCAGCTATTGGACGC	TGGCACTCCCAAACATAATTTGA
Human-GAPDH	CTGGGCTACACTGAGCACC	AAGTGGTCGTTGAGGGCAATG
VEGF-A	AGGGCAGAATCATCACGAAGT	AGGGTCTCGATTGGATGGCA
CD31	AACAGTGTTGACATGAAGAGCC	TGTAAAACAGCACGTCATCCTT
Rat-GAPDH	ACAGCAACAGGGTGGTGGAC	TTTGAGGGTGCAGCGAACTT
Runx2	GAACCAAGAAGGCACAGAC	AATGCGCCCTAAATCACTG
ALP	AGATGGATGAGGCCATCGGA	CCAAACGTGAAAACGTGGGA
OCN	CCCTCTCTCTGCTCACTCTGCT	CTTACTGCCCTCCTGCTTGG
OPG	CACGAGCCTTATCCCATTTGTAG	CTACCATAACCTACCCCTGCTTGT