ACCSCIENCE PUBLISHING

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RESEARCH ARTICLE

3D-printed zinc/magnesium-doped hydroxyapatite-polycaprolactone composite scaffolds for angiogenesis and osteogenesis

Supplementary File

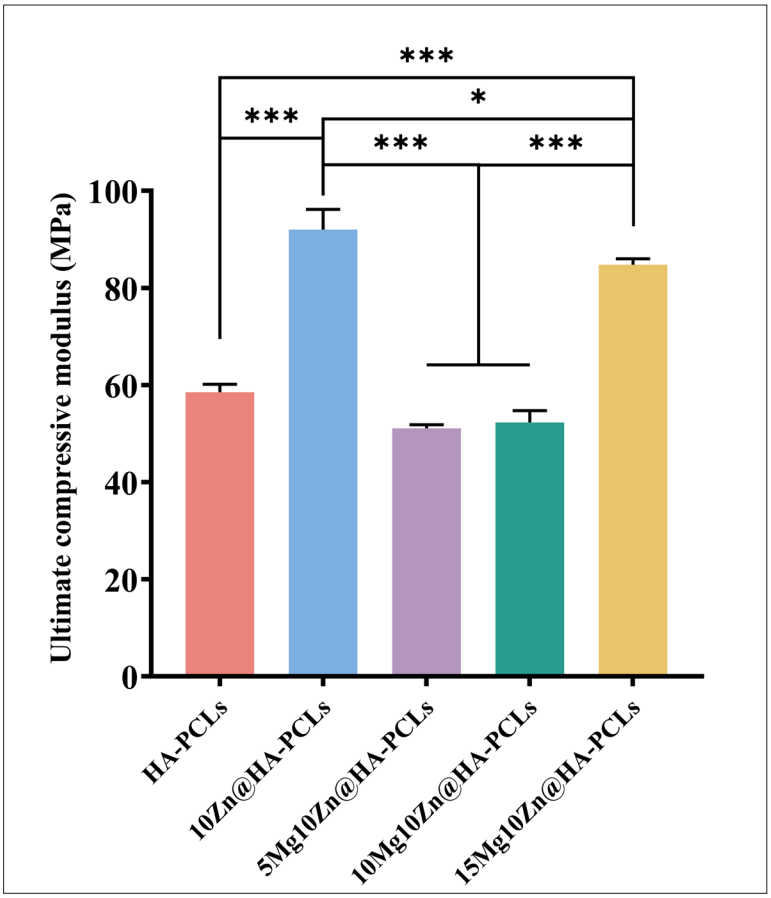

Figure S1. Ultimate compressive modulus of each group of scaffolds (n = 4). *p < 0.05, ***p < 0.001. Abbreviations: HA, hydroxyapatite; PCL, polycaprolactone.

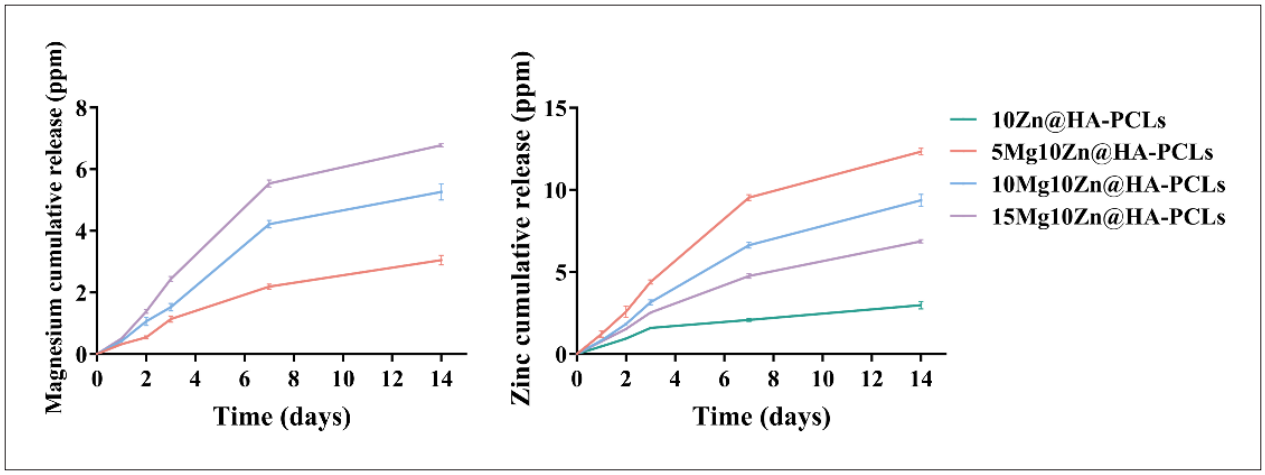

Figure S2. The release of magnesium (left) and zinc (right) in phosphate-buffered saline (PBS) over 14 days (n = 4). Abbreviations: HA, hydroxyapatite; PCL, polycaprolactone.

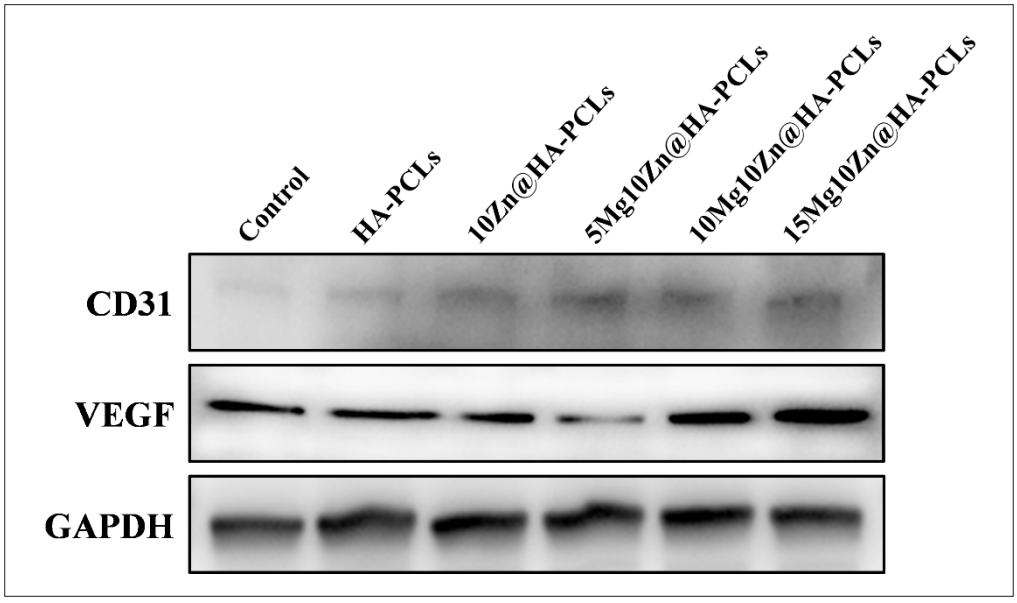

Figure S3. Angiogenesis-related protein in human umbilical vein endothelial cells (HUVECs), including CD31 and VEGF, at 24 h. Abbreviations: HA, hydroxyapatite; PCL, polycaprolactone.

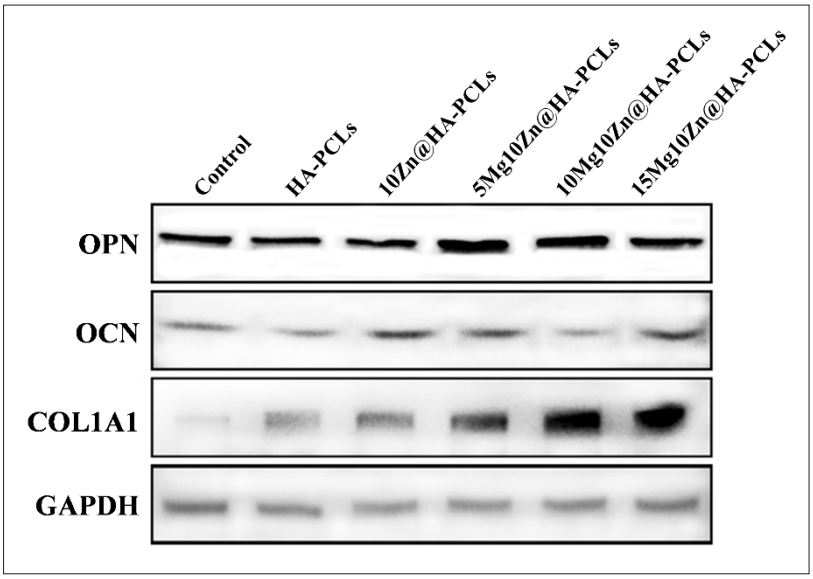

Figure S4. Osteogenesis-related protein in bone marrow mesenchymal stem cells (BMSCs), including OCN, OPN, and COL1A1, at 14 days. Abbreviations: HA, hydroxyapatite; PCL, polycaprolactone.

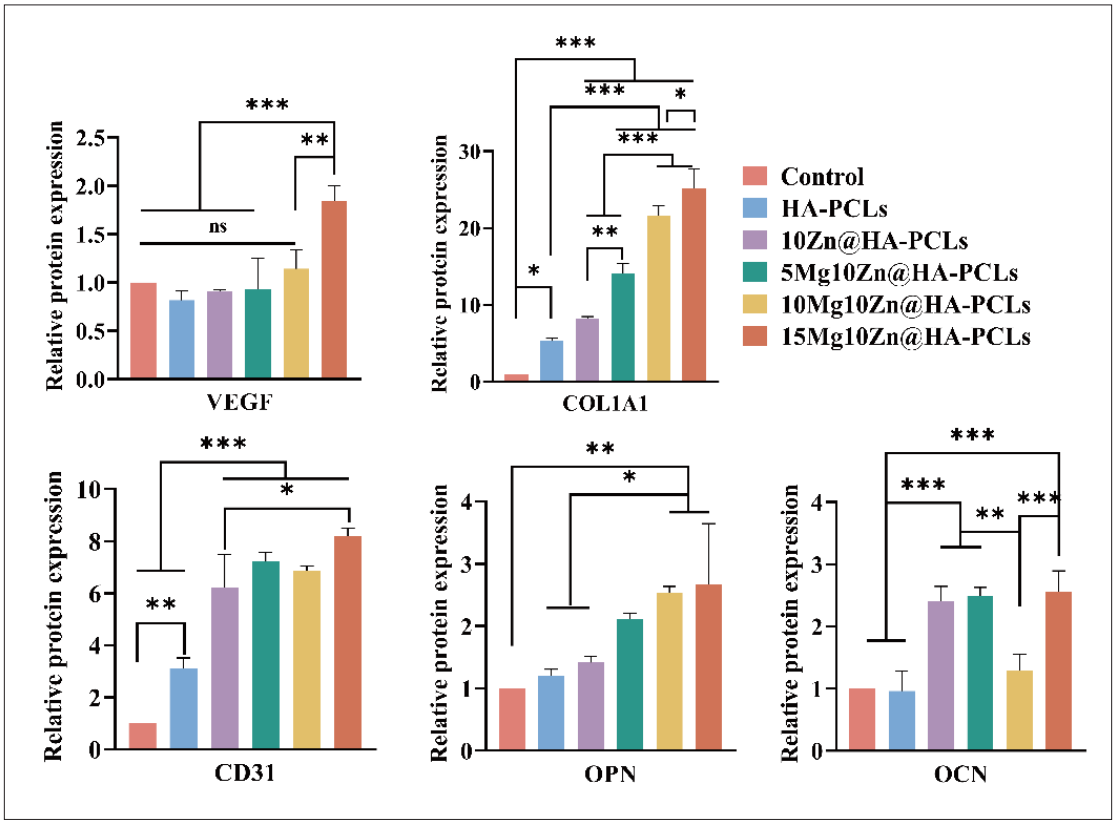

Figure S5. Quantitative analysis (relative protein expression) of western blot results for angiogenesis-related proteins CD31 and VEGF at 24 h and osteogenesis-related proteins OCN, OPN, ALP, RUNX2, and COL I at 14 days. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; $n = 3$. Abbreviations: HA, hydroxyapatite; ns: non-significant; PCL, polycaprolactone.

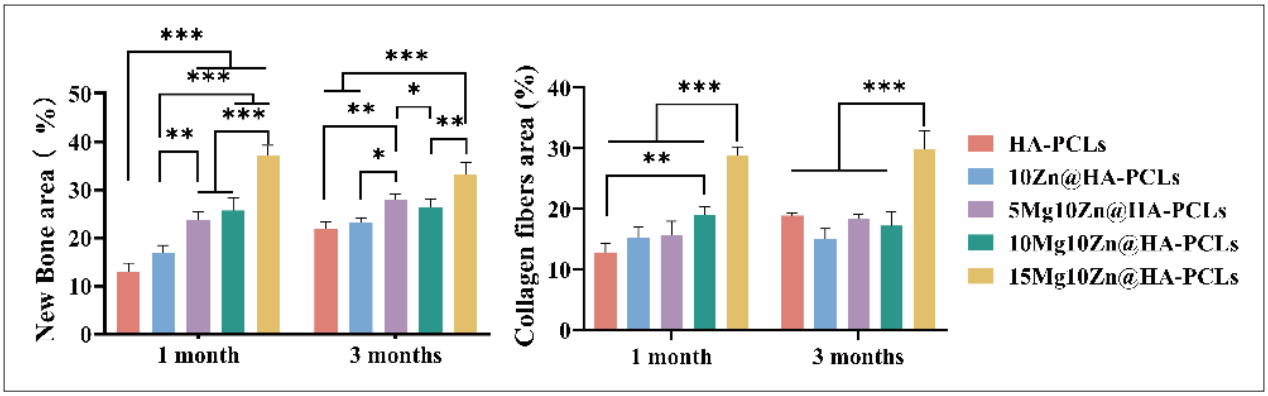

Figure S6. Quantitative analysis of hematoxylin and eosin (H&E; left) and Masson (right) staining at 1 and 3 months after composite scaffold implantation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; $n = 4$. Abbreviations: HA, hydroxyapatite; ns, non-significant; PCL, polycaprolactone.

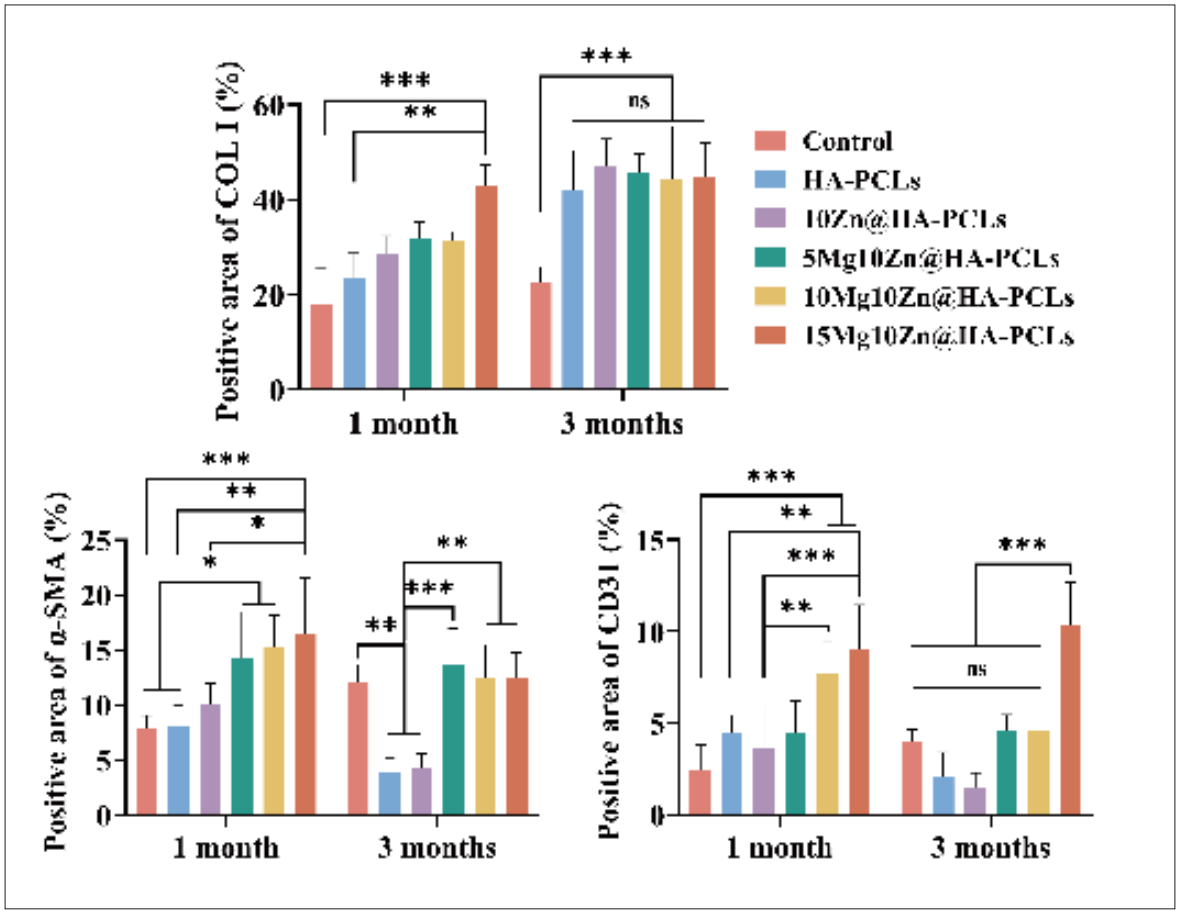

Figure S7. Quantitative analysis of α-SMA, CD 31, and COL1A1 in the newly formed tissues at 1 and 3 months after composite scaffold implantation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; $n = 4$. Abbreviations: HA, hydroxyapatite; ns, non-significant; PCL, polycaprolactone.

Table S1. Primers for reverse transcription quantitative polymerase chain reaction (qRT-PCR) analysis

Gene	Primer direction	Primer sequences (5'–3')
VEGF	Forward	CTACCTCCACCATGCCAAGT
	Reverse	CACACAGGATGGCTTGAAGA
FLT (VEGFR1)	Forward	GGGCTGAAACCATGTGCAAG
	Reverse	GCCAAAGATGCACTCCTCCT
KDR (VEGFR2)	Forward	GGCATGGGGTCTGTTCTGAA
	Reverse	TTGGCCAGGAGACACGTAAC
bFGF	Forward	AAAAGGCAAGATGCAGGAGA
	Reverse	TTTTGCAGCCTTACCCAATC
GAPDH	Forward	GTGGACCTGACCTGCCGTCTAG
	Reverse	GAGTGGGTGTCGCTGTTGAAGTC
OCN	Forward	GGACCCTCTCTCTGCTCACTCTG
	Reverse	ACCTTACTGCCCTCCTGCTTGG
OPN	Forward	GGGATCGCTGAAGGCATCAA
	Reverse	CAGCCGTCAAAGGCTTCAAA
RUNX2	Forward	TTTAGGGCGCATTCCTCATC
	Reverse	GGACTTGGTGCTGAGTTCA
COL I	Forward	CAGACCTGTGTGTTCCCTACT
	Reverse	TCTTTGCATAGCACGCCATCG
ALP	Forward	GCCTACTTGTGTGGCGTGAA
	Reverse	AGGATGGACGTGACCTCGTT
β-actin (Rat)	Forward	CATCCGTAAAGACCTCTATGCCAAC
	Reverse	ATGGAGCCACCGATCCACA

Table S2. Amount and efficiency of Zn and Mg

Sample	$\mathcal{X}_{t-Mg}$	$\mathcal{X}_{t-Zn}$	$\mathcal{X}_{a-Mg}$	$\mathcal{X}_{a-Zn}$	R_{Mg}(%)	R_{Zn}(%)
HA	0	0	0	0		
10Zn@HA	0	10	0	9.337		93.37
5Mg10Zn@HA	5	10	3.57	9.217	71.40	92.17
10Mg10Zn@HA	10	10	6.6	9.002	66.00	90.02
15Mg10Zn@HA	15	10	8.27	8.92	55.13	89.20

Abbreviations: HA, hydroxyapatite; R, relative doping; *X*a, actual doping; *X*t, theoretical doping.