Lithium-doped surface inspires immunomodulatory functions for enhanced osteointegration through PI3K/AKT signaling axis regulation

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1. Methods and Materials

1.1. Cell viability evaluation

The CCK-8 assay and Live/Dead staining were performed to evaluate the cytocompatibility of the materials. Briefly, the sterilized Ti, Ti-Li0, Ti-Li1, and Ti-Li2 samples were placed on 24-well plates and BMDMs and C3H/10T1/2 were seeded on the different specimens with a density of 5×10^4 per sample and incubated for 1 day. The specimens with adhered cells were rinsed twice with the phosphate buffer saline (PBS). 500 μL of the cell culture medium containing 10 % CCK-8 were added to each well and incubated for another 4 h. 100 μL of the solution were then transferred to a new 96-well plate and the values of OD_{570} nm were measured to study the mitochondrial activities of the cells on the specimens. For live/dead staining, 300 μL of PBS containing Calcein AM (2 μM) and ethidium homodimer-1 (EthD-1, 4 μM) were added to each well and incubated for 30 min. The living cells expressing green fluorescence were analyzed under a fluorescence microscope.

1.2. Primer sequences of the genes used in this study

Table S1. Primer sequences of the genes used in this study.

Genes	Forward sequence	Reverse sequence
GAPDH (human)	CAAGAGCACAAGAGGAAGAGAG	CTACATGGCAACTGTGAGGAG
GAPDH (mouse)	TTCCAGGAGCGAGACCCCACTA	GGGCGGAGATGATGACCCTTTT
HIF-α	TCTACCAGTTGCAGCCTGAC	GTTCCCTTCCTCCTTGATTT
VEGF	CAGGACATTGCTGTGCTTTG	CTCAGAAGCAGGTGAGAGTAAG
PDGF-BB	GCTCCGTCTACGCGTCC	GAATGGGATCCCCCTCGG
Runx2	GACTGTGGTTACCGTCATGGC	ACTTGGTTTTTCATAACAGCGGA
ALP	TCCGTGGGCATTGTGACTAC	TGGTGGCATCTCGTTATCCG
OCN	GGTAGTGAACAGACTCCGGC	GGCGGTCTTCAAGCCATACT
COL-1	GCTCCTCTTAGGGGCCACT	ATTGGGGACCCTTAGGCCAT
Integrin α5	AGCAACTGCACCTCCAACTACA	ACACTTGGCTTCAGGGCATT
Integrin αv	AGACGTTGGGCCTATTGTTCA	ATTGGCCCGTCAATGTCGTA
Integrin β1	TTGGTCAGCAACGCATATCTG	CAGCAAAGTGAAACCCAGCAT
Integrin β3	ACCACACGAGGCGTGAACTC	CTCAATAGACTCTGGAGCACAATTG
FAK	GCAATGGAACGAGTATTAAAGGTCTT	GGCCACGTGCTTTACTTTGTG
Vinculin	GTGGCGACGGCACTACAGA	ACCGACTCCACGGTCATCTACT
Talin	CTTCCTGCAGTCTCCGCTAC	GGCCTGGCGATACCTGC
Paxilin	GACGACCTCGATGCCCTG	GCACCGCAATCTCCTGGTAT
TNF-α	GGACTAGCCAGGAGGGAGAA	CGCGGATCATGCTTTCTGTG
IL-6	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
iNOS	GGTGAAGGGACTGAGCTGTT	ACGTTCTCCGTTCTCTTGCAG
CCL-22	CTCTGCCATCACGTTTAGTGAA	GACGGTTATCAAAACAACGCC
IL-10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
Arg	CTCCAAGCCAAAGTCCTTAGAG	GGAGCTGTCATTAGGGACATCA
PI3K	CCAAGGAACCGGGACAGC	CACTCTCAGCTTCACCTCCG
mTOR	TTCCTGAACAGCGAGCACAA	GTAGCGGATATCAGGGTCAGG
AKT	TAGGCCCAGTCGCCCG	GCCAGGTTTTAATATATTCCCCTCG
PDK1	GTCGGAGCTTGGCGTTCC	AGCATTCACTGACCCGAAGT
Raptor	GTCGCCTCTTATGGGACTCG	CACTCACCGTCTTCATCCGA
PTEN	TCAAGAGGATGGATTCGACTTAGAC	ATGTCTCTCAGCACATAGATTGT

2. Results

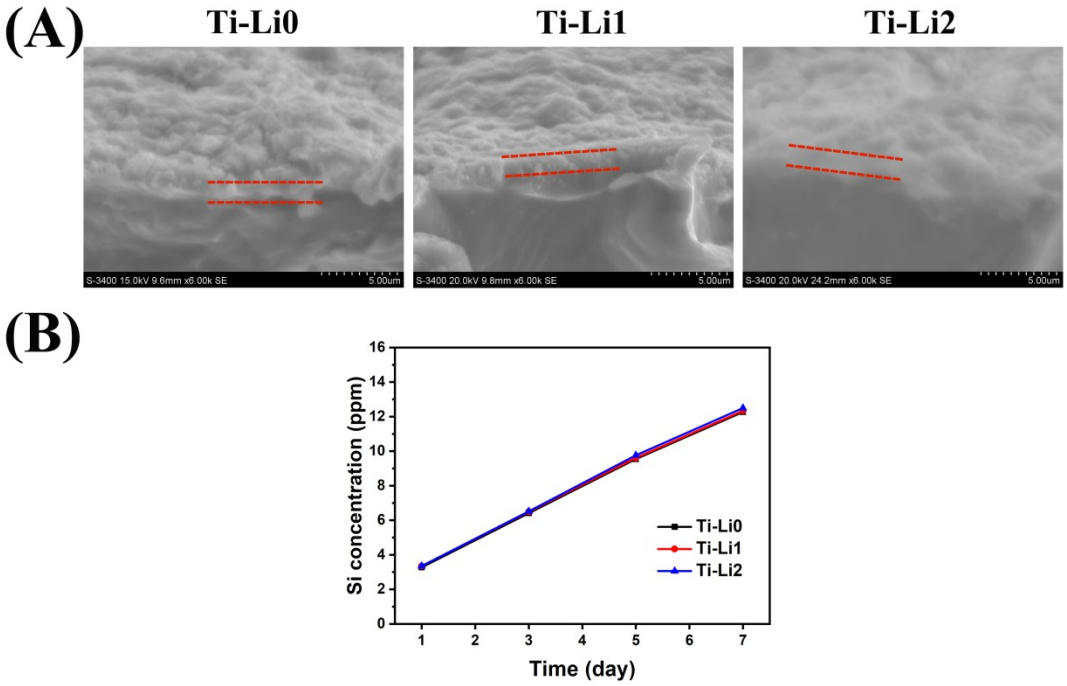

Figure S1. Cross-section images of PEO-coated samples (A); Cumulative release of Si ions from the PEO-coated samples incubated in deionized water for 7 days (B).

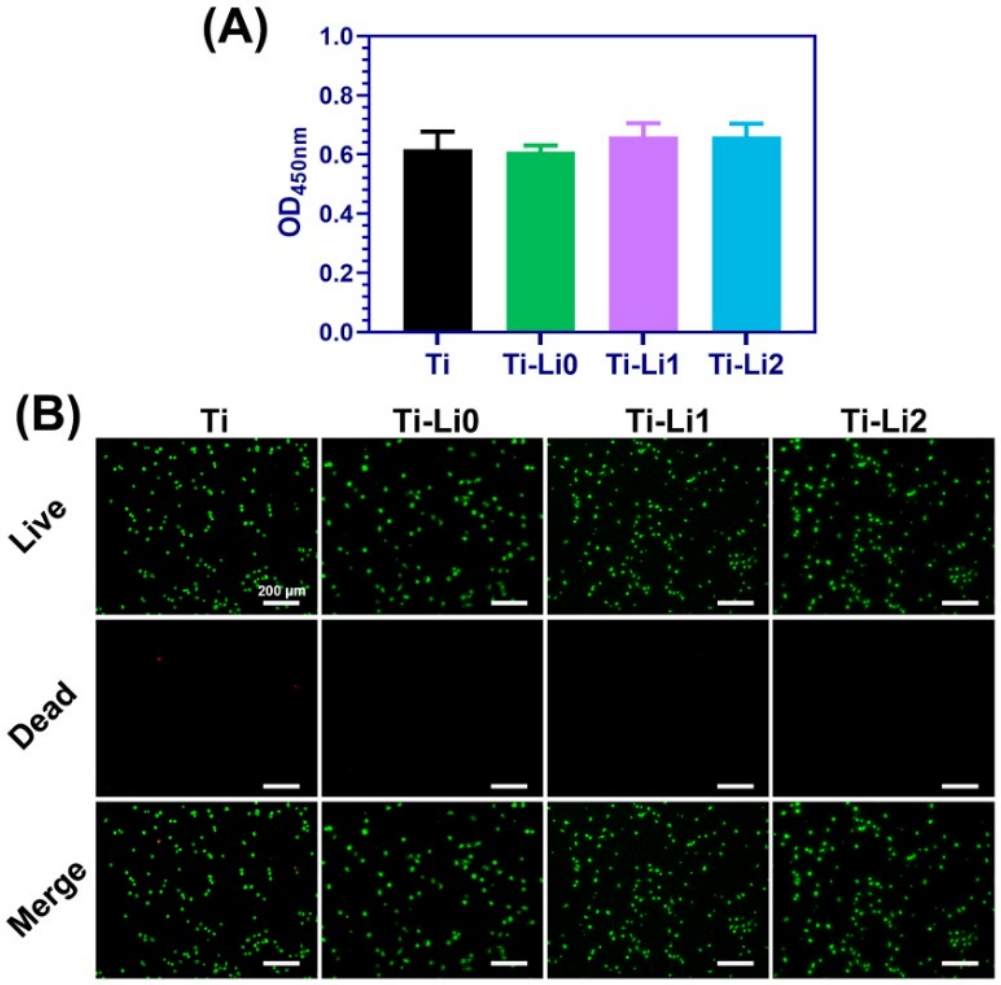

Figure S2. Proliferation (A) and Live/dead staining (B) of BMDM cultured on the samples for 1 day.

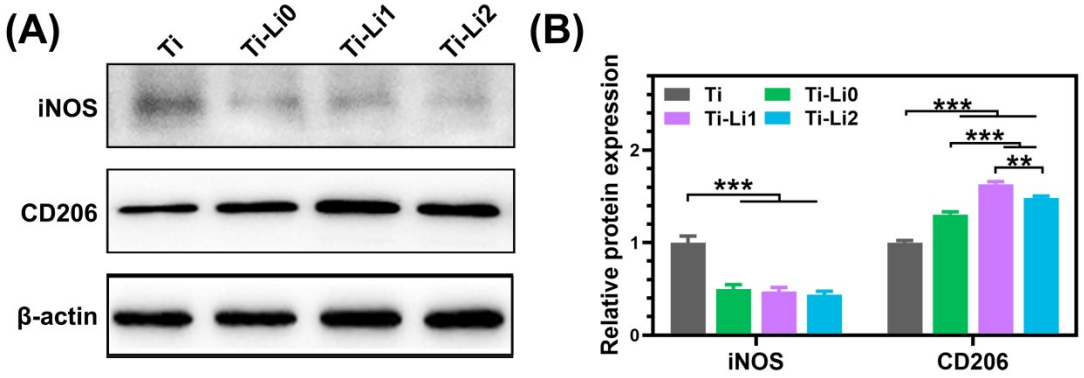

Figure S3. Qualitative (A) and quantitative (B) expression of iNOS and CD206 proteins in BMDM cultured on the samples for 1 day.

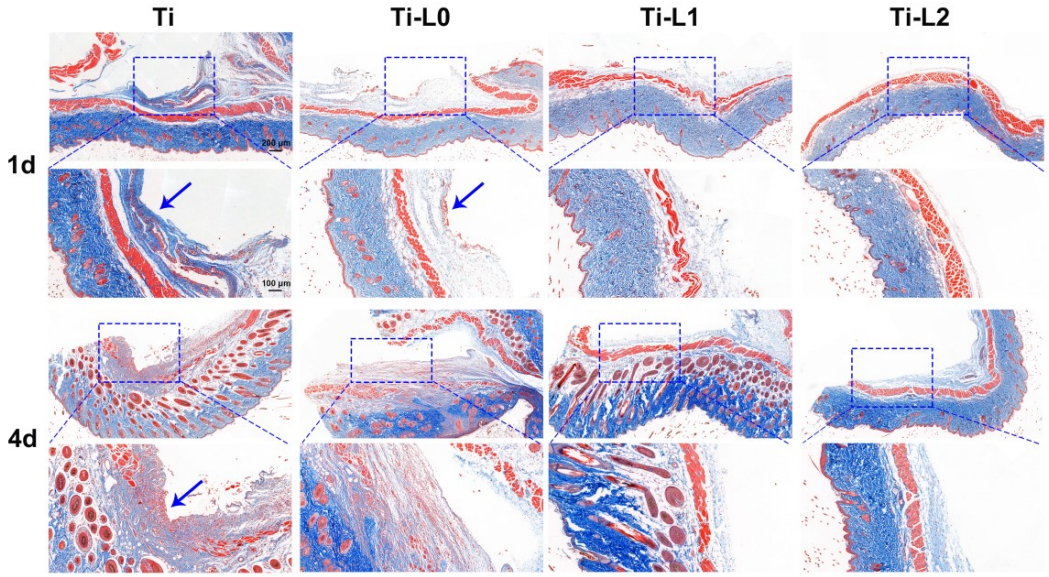

Figure S4. Masson staining of the tissues adjacent to the implants one and four days after implantation.

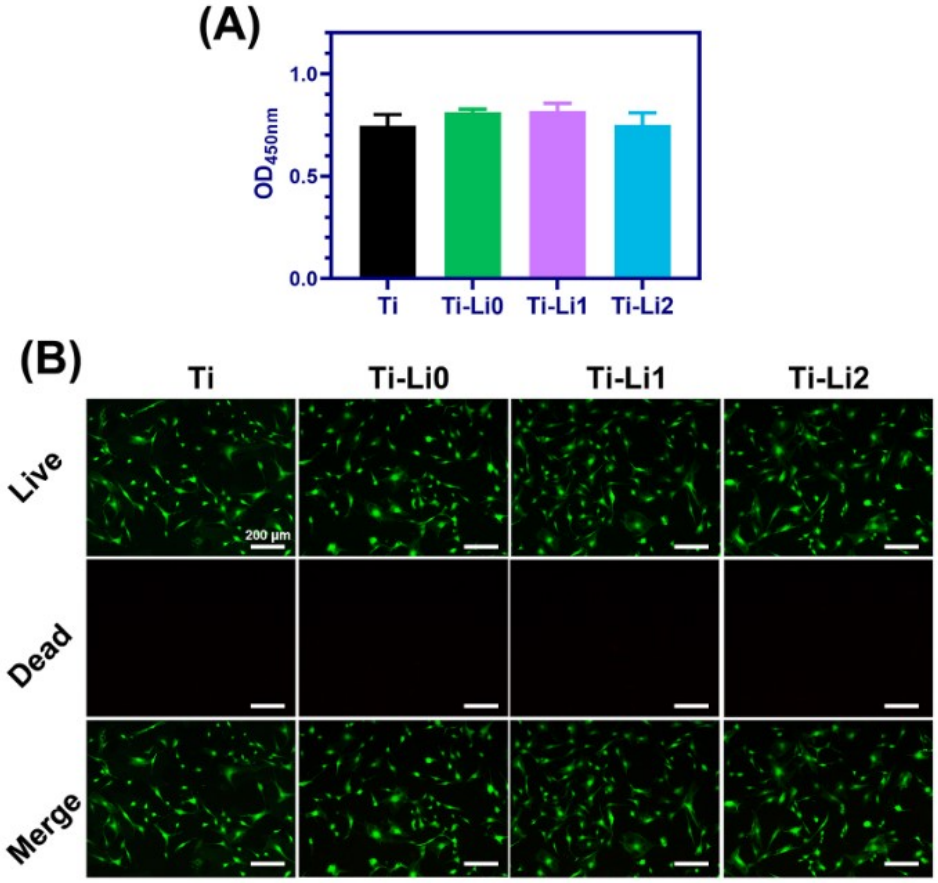

Figure S5. Proliferation (A) and Live/dead staining (B) of C3H10T1/2 cultured on the samples for 3 days.

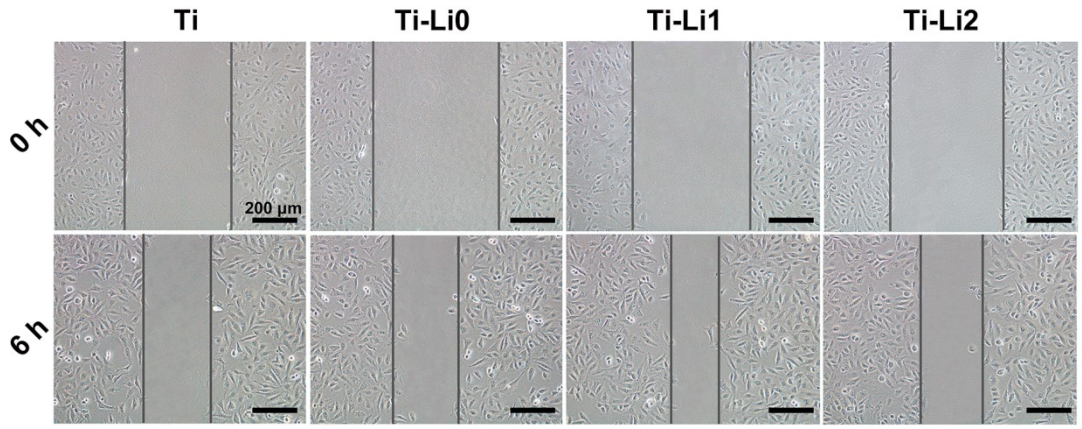

Figure S6. Migration of HUVECs cultured in various MCM for 6 h.

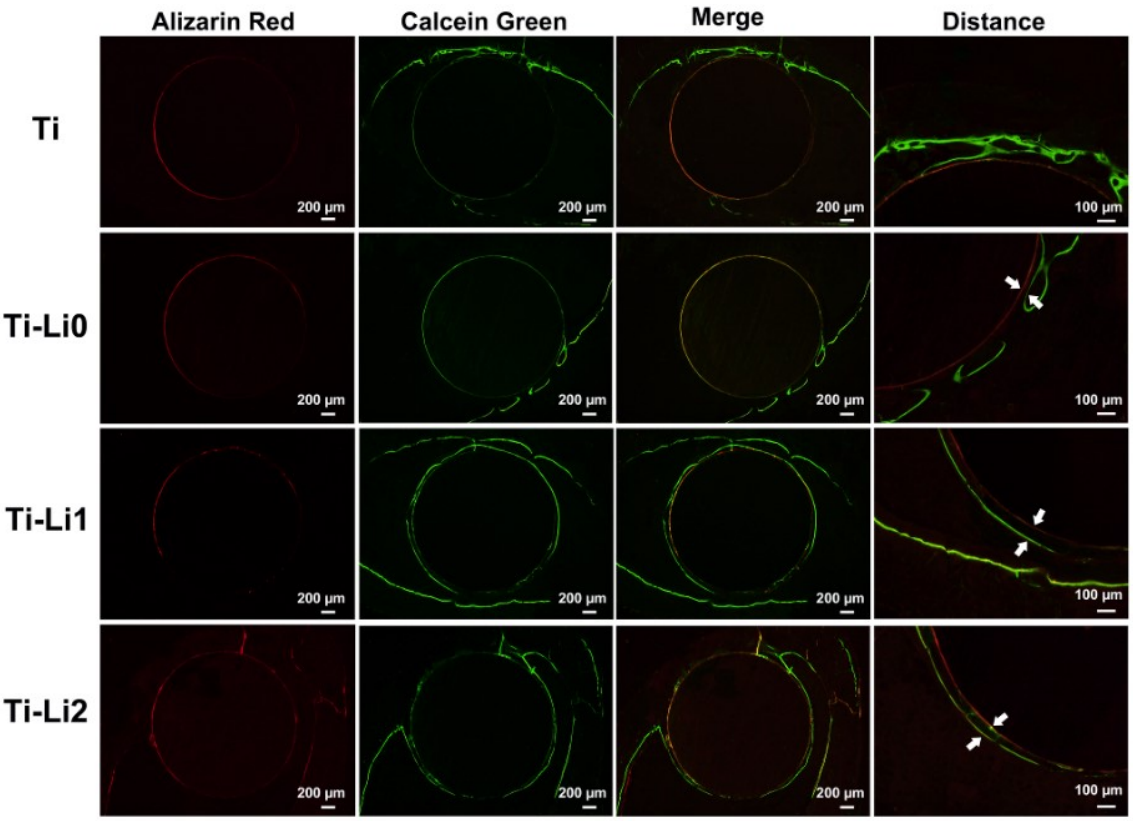

Figure S7. Sequential fluorescence labels for bones: red (Alizarin red, injected at week 4) and green (Calcein green, injected at week 6).

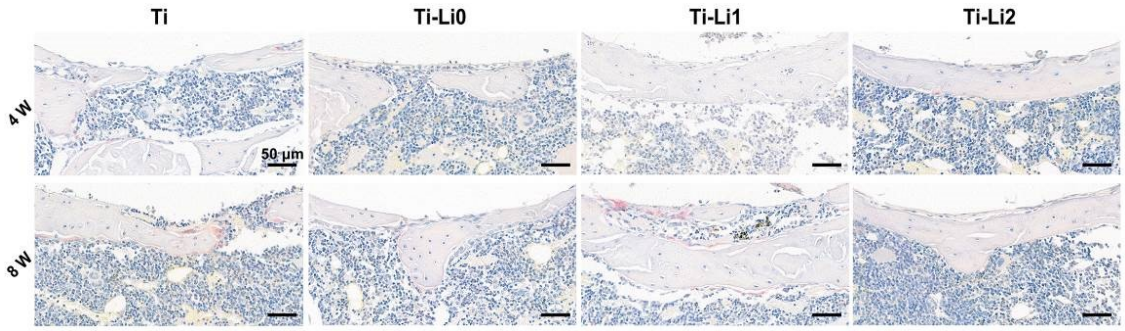

Figure S8. Immunohistological analysis of TRAP in the decalcified sections after 4 and 8 weeks.