

Periostin+ macrophages improved long bone regeneration in a mechanosensitive manner

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INTRODUCTION: Bone nonunion is an urgent orthopedic clinical problem with huge social burden [1]. Previous research found that a mechanosensitive protein, periostin, was expressed by macrophages within the new bone tissue [2]. Mechanical stimulation has been demonstrated to enhance the expression of periostin, consequently promoting bone repair [3], but the mechanism of periostin production in macrophages during bone repair is unclear. Therefore, we speculate that mechanical stimulation promotes bone tissue regeneration by regulating the expression of periostin in macrophages.

METHODS: Monocortical tibial defects (MTD) were created in C57BL/6J (WT), Lysm-Cre; tdTomato (tdTomato) and Lysm-Cre; POSTN^{-/-} (cKO) mice. Tibiae of tdTomato mice were collected on post-surgical day (PSD) 8, 10, and 14. Compressive axial dynamic mechanical loading (~1800 μ e peak, 2 Hz sinusoidal, 120 cycles/day) was applied to the left tibia of WT mice or cKO mice on PSD 5 to 8. Mice were sacrificed on PSD 10 and tibiae were collected. The expression of periostin in macrophages were assessed by colocalization and spatial analysis of immunofluorescence data. New bone formation within the tibial defect was measured by Micro-CT and histology staining. Mechanical strain concentration was measured by digital image correlation (DIC) and simulated by finite element analysis (FEA). Finally, we transplanted mechanically-conditioned bone marrow-derived macrophages (BMDMs) to the MTD with the surrounding periosteum removed.

RESULTS: Large numbers of macrophages expressed periostin from PSD 3 to PSD 14, with higher number on PSD 10 and 14, as observed by colocalization of macrophages and periostin (Figure 1a, b). Higher number of M2 macrophages co-localized with periostin compared with M1 macrophages (Figure 1c). Periostin expressed in a spatially-correlated with a strain-dependent distribution, which was high concentrated in the defect site (Figure 1d). On PSD 10, cKO mice had lower bone accrual compared with WT, while mechanical loading induced higher bone volume and trabecular number in cKO mice (Figure 1e). Transplantation of FSS- or TGF- β -treated BMDMs induced higher expression of OSX+ cells, EMCN+ vessels and periostin compared to non-conditioned BMDMs, while no differences have been observed between FSS- and TGF- β -treated BMDMs (Figure 1f, g).

DISCUSSION: We uncovered a significant role of macrophage-derived periostin in the repair of bone defects. Periostin functioned as a key effector protein by macrophages, particularly M2 subtype in response to mechanical stimuli. Mechanical stimulation exerted a dual effect, inducing M2 macrophage polarization while concurrently facilitating osteoblast differentiation. Consequently, this signaling axis expedited the process of bone healing in response to mechanical stimulation.

SIGNIFICANCE: These findings demonstrated periostin expression by macrophage as a mechanosensitive phenomenon, and had significant impact on bone regeneration, highlighting the potential utility of mechanically-conditioned macrophages in orthopedic applications.

REFERENCES: [1] Yang N., et al. Int J Med Sci, 2021; [2] Wang Z., et al. FASEB J, 2021; [3] Liu C., et al. J Bone Miner Res, 2019.

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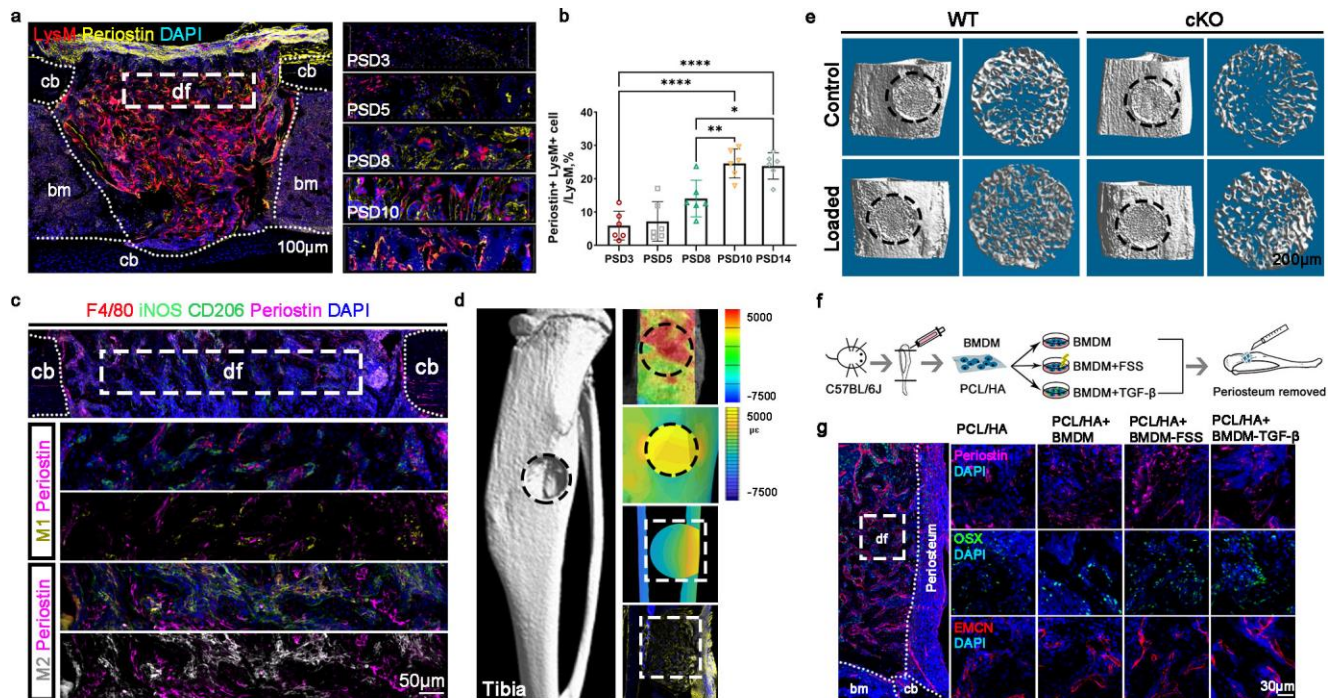


Figure 1. Mechanical loading migrated the poor bone regeneration caused by periostin deletion in macrophages. a,b Macrophages expressed periostin during bone regeneration. **c** Periostin was primarily expressed by M2 macrophages in the bone defect. **d** Periostin expressed in a strain-dependent way. **e** Mechanical loading mitigated the impaired bone formation caused by periostin-deletion. **f** Schematic for transplantation macrophages at the defect site during. **g** Mechanically-conditioned macrophages increased the expression of periostin, OSX+ cells and vessels. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Data were mean \pm SD.