Magnesium-containing Implant Modulates the Characteristics of Distinct Mesenchymal Progenitors to Inhibit Fracture Callus Fibrosis in Long-term Bisphosphonate-pretreated Rats

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INTRODUCTION: Fracture callus fibrosis was found to be the key pathologic change in rats receiving long-term bisphosphonates (BPs) pre-treatment, which recapitulates the impaired fracture healing in atypical femoral fracture (AFF) patients with long-term BPs use clinically. Besides, dysfunction of specific mesenchymal progenitors has been demonstrated to play key roles in fibrosis-associated fractures, such as polytraumatic, radiation-associated, and diabetic fractures. Thus, the present study aims to investigate the anti-fibrotic effects of Mg-containing implants (MCI) in long-term BPs pretreatment-impaired femoral fracture healing in rats at single-cell resolution.

METHODS: In this work, we used single-cell transcriptome sequencing (scRNA-seq) to depict the cellular atlas of fracture callus cells (FCCs) at 4 and 12 weeks post-fracture in Ctrl, BP, and BP-Mg groups respectively. The anti-fibrosis effects of Mg-containing implants and the validation of sequencing results were conducted in vivo and in vitro by performing immunofluorescence, flow cytometry, differentiation assay, real-time PCR, etc.

RESULTS: We found that there were no significant differences in transcriptomes among Ctrl, BP, and BP-Mg groups at 4 weeks post-fracture, suggesting the relatively normal fracture healing process at the early stage in both BP and BP-Mg groups. However, as fracture healing progressed (12wpf), the expression of fibrotic markers, such as Col1a1, Col3a1, Fn1, Acta2, and Tgfb1, was dramatically upregulated in BPs-treated rats while decreased by implantation of MCI (Fig. A). At the cellular level, two subsets of mesenchymal progenitors were defined, one was Grem1$^+$ CD105$^+$ CD90$^+$, and another was Prx1$^+$ CD90$^+$. Interestingly, Grem1$^+$ mesenchymal progenitors were dramatically increased in the BP-Mg group at 12wpf (Fig. B), accompanied by activation of the chemokine signaling pathway. In vitro experiments demonstrated that Prx1$^+$ FCCs possessed greater myofibrogenic differentiation potential, while Grem1$^+$ FCCs possessed higher osteogenic differentiation potential. Furthermore, BPs pre-treatment augmented the myofibrogenic potential of both Grem1$^+$ and Prx1$^+$ FCCs, while reducing the osteogenic potential of Grem1$^+$ mesenchymal progenitors. By comparison, the implantation of MCI alleviated the pro-fibrotic effects of BPs on both Grem1$^+$ and Prx1$^+$ FCCs, while rescuing the attenuated osteogenic potential of Grem1$^+$ FCCs obtained from BPs-treated rats.

DISCUSSION: We demonstrated that MCI inhibited fracture callus fibrosis in long-term BPs-pretreated rats via differential modulation of Grem1+ and Prx1+ mesenchymal progenitors for the first time (Fig. C). Our study will shed new light on the potential development and application of Mg-containing devices in challenging musculoskeletal disorders associated with aberrant fibrosis.

SIGNIFICANCE/CLINICAL RELEVANCE: The present study explored the underlying mechanisms of long-term BP pretreatment-impaired fracture healing and the anti-fibrotic effects of Mg-containing intramedullary nails at single-cell level, highlighting the key roles of different mesenchymal progenitors in the aberrant fracture healing process. Our findings will push forward the potential development and application of Mg-containing devices in challenging musculoskeletal disorders associated with aberrant fibrosis, such as atypical femur fractures, radiation-associated fractures, and diabetic fractures.

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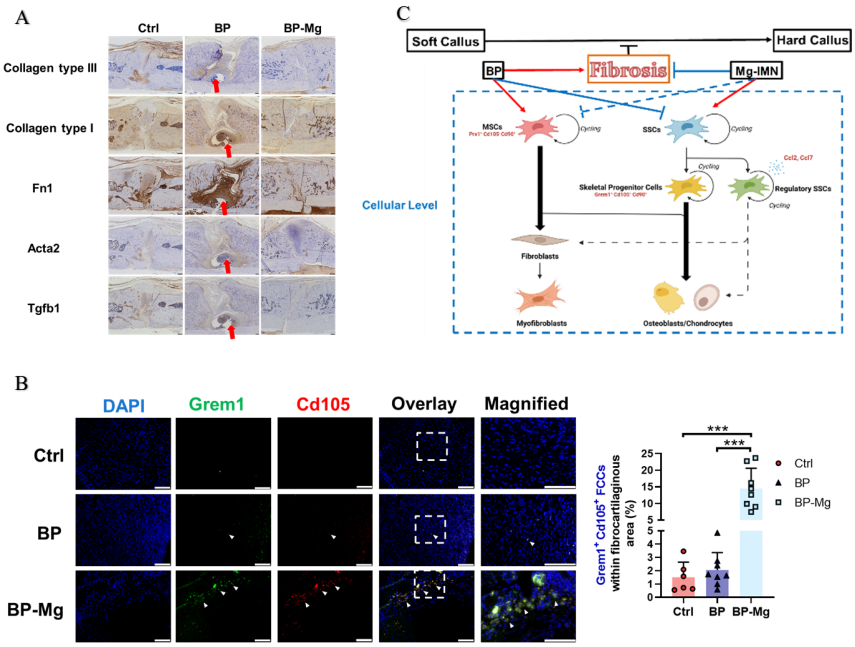

Figure A: Validation of the expression of multiple fibrotic markers in callus of Ctrl, BP, and BP-Mg group at 12wpf by immunohistochemistry. Red arrows indicated the center of the fibrosis region within the fracture gap. Scale bar = 200 μm.
Figure B: (Left panel) Validation of the Grem1$^+$ Cd105$^+$ FCCs in Ctrl, BP, BP-Mg groups at 12wpf by immunofluorescence. White arrowheads indicated Grem1$^+$ Cd105$^+$ mesenchymal progenitors. White dotted boxes indicated the ROI that was magnified. Scale bar = 100 μm. (Right panel) Quantitative analysis of the percentage of Grem1$^+$ CD105$^+$ FCCs within the fibrocartilaginous area in fracture callus at 12wpf. Quantitative data was presented as mean ± SD. One-way ANOVA with Tukey's multiple comparisons test was used to compare data between each group. Significant difference was defined as: *p < 0.05, **p < 0.01, and ***p < 0.001.
Figure C: The schematic diagram shows that MCI inhibited fracture callus fibrosis in long-term BPs-pretreated rats via differential modulation of Grem1$^+$ and Prx1$^+$ mesenchymal progenitors.