EchAMP-functionalized scaffold eliminates infection and promotes bone regeneration via immunometabolic reprogramming

Peng Ye [1,2], PhD ; Xiaoqian Pan[1]，MD；Jie Liu[1]，MD；Jing Din[2]，MD；Yajun Liu[2], PhD；Qian Chen[2], PhD
[1]Affiliated Hospital of Zunyi Medical University, Zunyi, Guizhou，China [2] Department of Orthopaedics, Warren Alpert Medical School of Brown University/Rhode Island Hospital, Providence, RI，USA
Email:peng_ye@brown.edu

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INTRODUCTION: Addressing the core dilemma of infectious bone defects—persistent infection, M1-driven inflammation, and impaired bone repair—we fabricated and characterized a porous scaffold for bone healing functionalized with EchAMP, an anti-microbial peptide. We systematically evaluated in vivo properties of the scaffold including its antibacterial, immunoregulatory, and osteoregenerative effects, and explored potential mechanisms through integrated proteomics and metabolomics[1–3].

METHODS: A porous scaffold was constructed by freeze-drying and loaded with EchAMP. Materials characterization included physicochemical structure (SEM, pore size/porosity, XRD, FTIR), mechanical properties (compressive modulus), drug loading and release profiles, as well as in vitro antibacterial activity and cytocompatibility[4]. A Staphylococcus aureus–contaminated long-bone defect model was established with three groups: infection control, blank scaffold, and EchAMP scaffold. At 1 and 4 weeks postoperatively, we performed micro-CT morphometry; H&E, TRAP, and Giemsa staining; F4/80+iNOS/CD206 immunofluorescence with quantification; and, at week 4, KEGG enrichment analyses based on proteomics and metabolomics[5].

RESULTS SECTION: Materials characterization showed a well-interconnected porous architecture with controllable release and good biocompatibility. In vivo at week 1, EchAMP markedly reduced bacterial burden and acute suppurative responses, significantly decreased M1 and increased M2 polarization ($P < 0.001$), and downregulated pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-17A) (Fig. 1A). By week 4, the EchAMP group exhibited continuous woven/lamellar bone and canaliculi-like morphology. Micro-CT revealed increased BMD, BV/TV, Tb.Th, Tb.N, and Ct.Th with reduced Tb.Sp; osteoblasts increased and TRAP+ osteoclasts decreased (all $P < 0.05$), whereas improvements in the control and blank groups were limited and not significant (Fig. 1B). Multi-omics findings were consistent with histology: EchAMP downregulated pathogen-associated pathways and lysosome/phagosome and complement–coagulation cascades, while upregulating mitochondrial energy metabolism (TCA cycle, oxidative phosphorylation, coenzyme Q biosynthesis), glycolysis–pyruvate flux, and nucleotide and amino acid metabolism (arginine, branched-chain/aromatic) (Fig. 1C).

DISCUSSION: The EchAMP-functionalized scaffold establishes a clear cascade—rapid bacterial clearance, immunometabolic reprogramming with M1→M2 shift, and restoration of bone formation–resorption balance[6]. Early M1↓/M2↑ with cytokine suppression indicates disruption of inflammatory positive feedback; late continuous woven/lamellar bone with comprehensive gains in micro-CT metrics, increased osteoblasts, and fewer TRAP+ osteoclasts suggests active regeneration beyond mere infection relief[7,8]. Multi-omics mechanistically supports this cascade, linking dampened pathogen/complement axes with heightened TCA/OXPHOS, coenzyme-Q biosynthesis, glycolysis–pyruvate flux, and arginine metabolism—metabolic signatures consistent with M2 programming and matrix production. Overall, the EchAMP scaffold enables integrated "infection control plus regeneration" for infectious bone defects.

SIGNIFICANCE/CLINICAL RELEVANCE: In high-risk infectious bone defects, EchAMP-loaded scaffolds provide localized antibacterial protection and restore bone formation–resorption balance via M1→M2 immunometabolic shift, offering a feasible adjunct or alternative to antibiotic cement with prospects for clinical translation.

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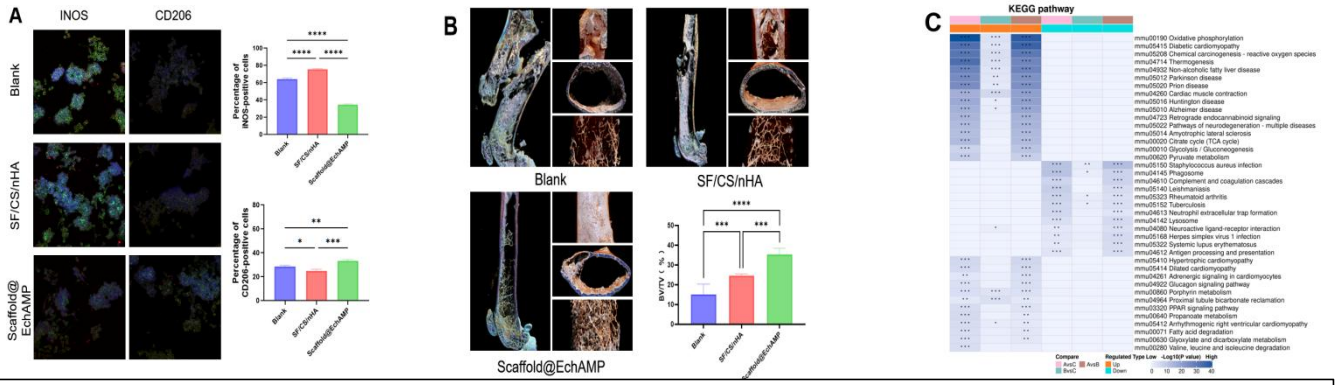

Figure 1A. Macrophage polarization by IF: iNOS and CD206 (green) with DAPI (blue) in Blank, Scaffold (SF/CS/nHA), and Scaffold@EchAMP (scale bar: 50 μm). iNOS is highest in Scaffold and lowest in Scaffold@EchAMP; CD206 is highest in Scaffold@EchAMP; **Figure 1B.** Bone regeneration: 3D and cross-sectional views of femoral defects for Blank, Scaffold, and Scaffold@EchAMP. BV/TV (%) increases from Blank < Scaffold < Scaffold@EchAMP; **Figure 1C.** KEGG enrichment heatmap: GroupA/GroupB/GroupC = Blank/Scaffold/Scaffold@EchAMP. Color shows− log10(adjusted p); darker = more significant.