Mitochondria-Targeted Single-Atom Nanozymes Reprogram Stem Cell Metabolism to Accelerate Bone Regeneration

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INTRODUCTION: Critical-sized bone defects (CSBDs) present significant orthopaedic challenges, with impaired stem cell function often underlying poor regenerative outcomes. Emerging evidence indicates that mitochondrial dysfunction driven by pathological redox imbalance critically compromises stem cell energy metabolism and osteogenic potential. While biomaterials addressing oxidative stress show promise, current strategies fail to target mitochondria for metabolism modulation during osteogenesis. We **hypothesized** that precisely augmenting stem cell bioenergetics through mitochondria-targeted nanozymes will overcome this limitation. Herein, we developed mitochondria-targeted single-atom Fe/Cu nanozymes to test whether: (1) direct potentiation of fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS) accelerates osteogenesis, and (2) concurrent ROS scavenging and autophagy activation sustains metabolic homeostasis in mitochondria, ultimately enhancing stem cell-based bone regeneration.

METHODS: (1) *Nanozyme Synthesis & Characterization*: TPP-DMSN-Fe/Cu nanozymes were synthesized via sequential coordination of Fe/Cu single atoms on dendritic mesoporous silica nanoparticles (DMSNs) followed by triphenylphosphonium (TPP, for targeting mitochondria) modification (**Fig. 1A**). Physicochemical properties were characterized by TEM, SEM, and EDS (**Fig. 1B-E**). (2) *In Vitro Studies*: Murine stem cells (C3H10T1/2, Clone 8; Cell Bank/Stem Cell Bank, Chinese Academy of Sciences, Catalog #: SCSP-506) were cultured in osteogenic media with or without the nanozymes (10 μg/mL). Differentiation (7/14 days) was assessed by ALP/ARS staining, qPCR (*Runx2*/*Ocn*/*Opn*/*Alp*), RNA sequencing, metabolic flux analysis (Seahorse XFe96), mitochondrial biogenesis, and autophagy flux (AMPK/ PGC-1α and LC3-II/Beclin-1 Western blot). (3) *In Vivo Tests*: CSBDs (3 mm) in tibia were created in 12-week-old male Sprague-Dawley rats (n = 3/group), which received 1) no treatment (control), 2) GelMA scaffold, 3) DMSN + scaffold, 4) DMSN-Fe/Cu + scaffold, or 5) TPP-DMSN-Fe/Cu + scaffold. Rationale for animal sex selection: Male rats were selected to avoid estrogen-mediated metabolic variability while using an established bone repair model. Statistical analysis: Data are presented as mean ± SD. Multi-group comparisons: one-way ANOVA + Tukey's test (GraphPad Prism 10; n ≥3 biological replicates for all tests). Ethics: The animal experiments were authorized by the Animal Experimental Committee of the Seyotin (Guangdong) Co., Ltd (Approval SYT2024068) and carried out in accordance with the regulations outlined in the National Law on Experimental Animal Utilization (P.R. China).

RESULTS: *In vitro*, TPP-DMSN-Fe/Cu nanozymes profoundly reprogrammed energy metabolism in C3H10T1/2 cells, elevating FAO-driven OXPHOS (**Fig. 2A-C**). Concomitantly, osteogenic differentiation was augmented, evidenced by upregulation of *Runx2*, *Ocn*, *Opn,* and *Alp* mRNA (**Fig. 2D**), alongside enhanced autophagy, *p*-AMPK/AMPK signaling, and PGC-1α expression to promote mitochondrial biogenesis (**Fig. 2E**). *In vivo*, TPP-DMSN-Fe/Cu nanozyme treatment of rat CSBDs achieved near-complete regeneration, with μCT revealing 2.8- (Week 4) and 4.1-fold (Week 8) increase in bone volume/total volume (BV/TV) versus the control (**Fig. 3A, B**) and immunostaining confirming robust bone regeneration by week 4 and 8 (**Fig. 3C**).

DISCUSSION: Our mitochondria-targeted TPP-DMSN-Fe/Cu nanozyme resolves metabolic impairment in stem cells, accelerating CSBD repair by coupling FAO-driven energy production with autophagy-mediated mitochondrial biogenesis. Although previously reported mitochondria-targeted polyphenol/amino acid NPs enhanced mitochondrial biogenesis and reducing ROS, these NPs lacked the capacity to drive the metabolic reprogramming required for osteogenesis. Our results highlight mitochondria-specific metabolic reprogramming as a therapeutic strategy for repairing complex bone defects. For clinical translation, large-animal validation is needed, and effects of the nanozymes on stem cell subpopulations need to be studied.

SIGNIFICANCE/CLINICAL RELEVANCE: This work addresses a critical barrier in CSBD treatment—mitochondrial dysfunction in stem cells—by introducing precision metabolic reprogramming (**Fig. 2A**) via mitochondria-targeted nanozymes. By augmenting osteogenesis with biocompatible, multifunctional nanozymes, our strategy effectively accelerates bone healing, holding immense potential for treating various bone disorders, including bone trauma, osteoporosis, and aging-related fractures, where dysregulated stem cell energy metabolism impedes repair.

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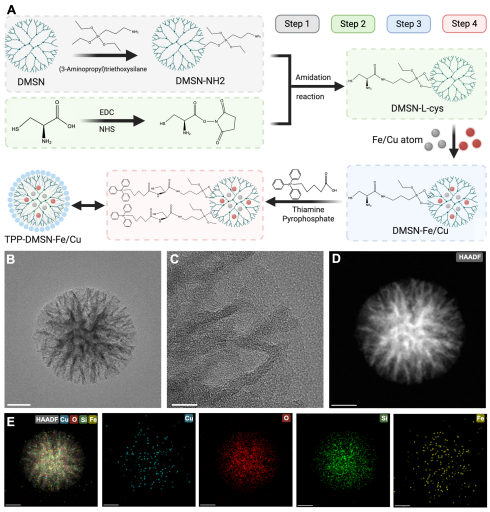

Fig.1 Synthesis and characterization of TPP-DMSN-Fe/Cu nanozymes. (A) Synthesis process. (B, C) TEM images. Scale bars = 50 nm (B) and 10 nm (C). (D) HAADF image showing the dendritic mesoporous structure. (E) EDS images. Scale bars = 50 nm.

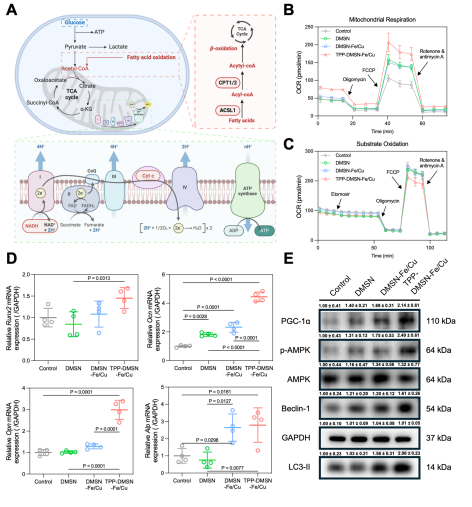

Fig. 2 Nanozymes regulated energy metabolism in stem cells. (A) Schematic diagram of metabolic regulation. (B, C) Real-time OCRs with mitochondrial stress (B) and substrate oxidation (C). (D) Osteogenic gene expression. (E) Western blot bands.

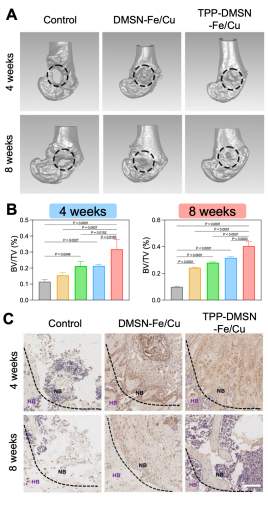

Fig. 3 Nanozymes for CSBD repair *in vivo*. (A, B) Reconstructed μCT images and quantitative analysis. (C) OCN IHC staining images. Scale bars = 100 μm.