Scaffold with high curvature pores promotes segmental bone defect repair by regulating skeletal stem cells

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INTRODUCTION: The reconstruction of large segmental bone defects remains a worldwide clinical challenge, but available treatment options for achieving biomechanically adequate restoration are currently limited. A common tissue engineering strategy to address this challenge involves the utilization of skeletal stem cells (SSCs) with scaffolds to mimic mature bone tissue [1]. During bone repair, mechanotransduction of SSCs plays a crucial role in mediating angiogenesis-osteogenesis coupling [2,3]. However, approaches to regulate SSCs' mechanotransduction for the repair of large bone defects have been lacking. In this study, we aimed to investigate whether mechanobiological optimization of pore curvature in 3D porous bioceramic scaffolds could promote segmental bone defect repair by regulating skeletal stem cells.

METHODS: In this work, we fabricated a mechanobiologically optimized 3D-printed biphasic calcium phosphate (BCP) scaffold. Two types of scaffold designs were used: octet truss for its high curvature corners and Kelvin cell for its low curvature corners. All animal procedures were approved by the Southern University of Science and Technology Animal Care and Use Committee. Experiments were performed on 16-week-old female C57BL6 mice (N=27) and $Prx1^{Cre}$; $Rosa^{tdTomato}$ mice (N=9). Subcutaneous implantation was performed to test biological compatibility. Scaffolds were implanted into 2 mm unilateral femoral segmental defects with external fixation. Defects without scaffolds were used as controls. At 4 and 8 weeks after surgery, the sites of segmental defects were isolated and total RNA extracted to perform bulk RNA-seq (MGI2000). Analysis was performed utilizing the 'DESeq2' R package. At 2, 4, and 8 weeks after surgery, femurs were imaged by micro-computed tomography (μ-CT); SSCs and angiogenesis-osteogenesis coupling markers were measured by immunofluorescence staining; collagen fibers were imaged with second harmonic generation (SHG). Data are presented as mean ± standard deviation and were statistically analyzed by 1- or 2-way ANOVA with Tukey's post-hoc comparison or Student's t-test (α=0.05). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Data were represented as mean ± s.e.m.

RESULT: We fabricated mechanobiologically optimized scaffolds and performed the subcutaneous implantation and femoral segmental implantation (Figure 1A, 2A). The 3D-printed BCP scaffolds with higher curvature (octet truss) promoted the recruitment of endogenous SSCs. Kelvin cell had significantly higher stress magnitude at areas of stress concentration than octet truss under 50 N axial loading, which indicates fatigue cracks are more likely to initiate in Kelvin cells, the octet truss had higher crack resistance and mechanical strength (Figure 1B, P < 0.01). After 2 weeks of subcutaneous implantation, octet truss scaffold had the highest Osterix (OSX) and type H vessel volume. Octet truss also had a higher number of perivascular OSX+ osteoblasts in proximity to type H vessels at the corner of the scaffolds (Figure 2C, P < 0.0001). After 2 weeks in a femoral segmental defect, octet truss group had more paired-related homeobox protein 1 (PRRX1) +, leptin Receptor (LepR) +, and Gli1 + cells within the defects. Furthermore, the orientation of collagen fibers was aligned with scaffold surface (Figure 2D). At week 4 and 8 (Figure 3A), the upregulated pathways in octet truss group were the ECM-receptor interaction, focal adhesion, regulation of actin cytoskeleton, Ras and Rap1 signaling pathway (Figure 3B), which indicates that high curvature pores enhanced the mechanosensing and mechanotransduction of cells in segmental defects.

DISCUSSION: We demonstrated that high pore curvature alone such as those in acute angles could increase the number of SSCs and promote segmental bone defect repair by promoting angiogenesis–osteogenesis coupling. Our findings suggest a promising approach to designing mechanobiologically optimized scaffolds to regulate SSCs' mechanotransduction to promote bone regeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrates a promising strategy to fabricate and engineer mechanical microenvironment of SSCs for bone defect healing and bone tissue engineering, potentially offering a personalized therapeutic to patients with impaired bone defect healing.

REFERENCES: [1] S.J. Weiss et al. *Dev Cell*, 2022; [2] Chen, J. et al. *Stem Cell Reports*, 2022; [3] Morrison, S. J et al., *Cell Stem Cell*, 2022.

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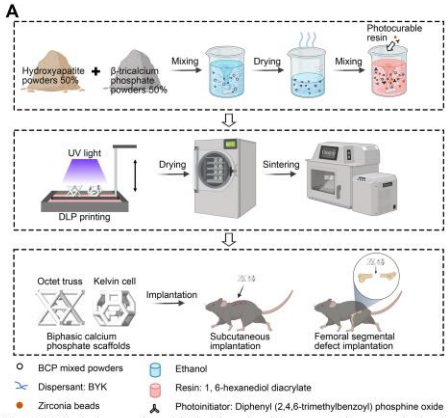

Fig. 1 (A) The hydroxyapatite and β-tricalcium phosphate nano-powders were mixed and dried in a 1:1 ratio to form BCP slurry, the photocurable resin and photoinitiator were added in the slurries to prepare for DLP printing. After printed, the BCP scaffolds were dried and sintered to form finally used bioceramic scaffolds. The unit length of octet truss and Kelvin cell is 2 mm, and the truss diameter is 0.3 mm, which could obtain cellular pores in the scaffolds. The subcutaneous implantation and femoral segmental defect implantation were performed to investigate the effects of BCP scaffolds on bone formation.

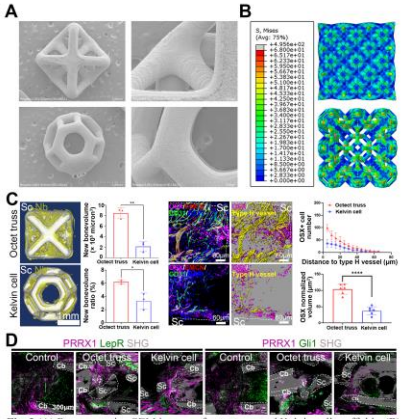

Fig. 2 (A) Representative SEM images of octet truss and Kelvin cell scaffolds. **(B)** The bulk modulus and yield strength of octet truss and Kelvin cell scaffolds. **(C)** Representative images and quantification results of μ-CT 2 weeks after subcutaneous implantation (scale bar = 60 μm); representative maximum intensity projections, 3D surface images and quantification results of OSX, EMCN, CD31 and DAPI immunofluorescence microscopy (scale bar = 60 μm) 2 weeks after subcutaneous implantation. **(D)** The immunofluorescence images of PRRX1, LepR, Gli1, and SHG in the segmental defects 2 weeks after implantation in femoral segmental defects. n = 3, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Sc: scaffold, Nb: new bone, Cb: cortical bone.

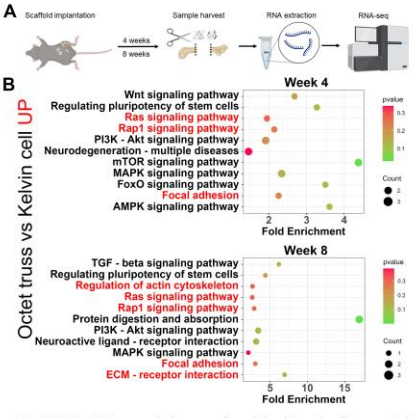

Fig. 3 (A) The RNA-seq analysis was performed 4 and 8 weeks after segmental defect implantation. **(B)** Compared to the Kelcin cell group, enriched KEGG pathways in the Octet truss group after 4, and 8 weeks of implantation. The horizontal axis represents the Rich factor. The enrichment factor indicates the number of differentially expressed genes belonging to a KEGG pathway/the total number of genes belonging to this KEGG pathway. The dot size represents the number of differentially expressed genes in a KEGG pathway, and the dot colour represents different p values.