Multi-gradient Osteochondral Unit-on-a-chip to Investigate Bone–Cartilage Crosstalk in Osteoarthritis

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INTRODUCTION: Osteoarthritis (OA), the most common joint disease, is characterized by dysregulated bone-cartilage crosstalk at the osteochondral (OC) interface. Articular cartilage and bone, while in close contact, are characterized by distinct extracellular matrix composition, structure, and function. Developing an effective OC tissue is crucial for studying OA mechanisms. However, engineering a physiologically relevant OC model poses significant challenges due to the complexity of simultaneously replicating the matrix stiffness, porosity, biochemical cues, and oxygen concentration. The emergence of organ-on-a-chip technology has greatly enhanced our ability to emulate human tissues *in vitro*. Herein, we hypothesize that a microfluidic osteochondral tissue-on-a-chip (OCoC) system with a patterned hydrogel can recreate the *in vivo* multi-gradient OC environment that supports active bone-cartilage crosstalk *in vitro*.

METHODS: The microfluidic chip consists of three layers and a porous membrane (**Fig. 1a**). Human mesenchymal stem cells were encapsulated in 15% and 7.5% GelMA hydrogels and injected into the left and right inlets of the bottom gradient-generating layer, respectively, to create stiffness and porosity gradients. The gradient hydrogel filled the bottom and middle layers. Normoxic (21% O_2) osteogenic medium and deoxidized (1% O_2) chondrogenic medium were injected into the left and right inlets the top gradient-generating layer, respectively, to realize oxygen and growth factor gradients. After 14 days, the proinflammatory cytokine interleukin (IL)-1β was added to the cartilage side over 48 h to induce OA-like cartilage inflammation and degeneration. The tissues from the chips were characterized by nanoidentation, immunostaining, PCR, and RNA sequencing. The medium effluents were analyzed by ELISA. Data analysis was conducted by Prism 10 ($N \geq 3$).

RESULTS SECTION: Both computer simulation and nanoindentation results supported the successful establishment of hydrogel scaffolds with native-like gradients in stiffness and porosity in the OCoC (**Fig. 1b, c**). A hypoxia-sensitive fluorescent probe showed the gradually decreasing oxygen concentration from bone to cartilage (**Fig. 1d**), which agreed with the simulation results (**Fig. 1b**). The PCR data showed gradually increasing expression levels for chondrogenic marker and *HIF1α* expression as well as decreasing osteogenic marker expression from bone to cartilage (**Fig. 1e**). These findings agreed with immunostaining, histology and ELISA results (**Fig. 1f, g**). Notably, ELISA and RNA-seq revealed significantly lower IL-1β-induced inflammatory responses in OCoCs with oxygen gradient than those in oxygen gradient-free chips perfused with normoxic media only (**Fig. 1g, 2a, b**). These data suggest the potential protective effects of oxygen gradient on joints against OA development. RNA-seq data revealed that such protective effects could be associated with the FGF18-FGFR3, Wnt/β-catenin, and TGFβ/Samd signaling pathways (**Fig. 2c**).

DISCUSSION: Compared with gradient-free, normoxic condition, the gradient oxygen environment promoted the formation of a native-like OC interface. Additionally, maintaining the hypoxic microenvironment for cartilage in the multi-gradient OCoC could effectively ameliorate OA-like degeneration.

SIGNIFICANCE: Our human cell-based, multi-gradient OCoC offers a highly physiologically relevant model for OA research and drug development.

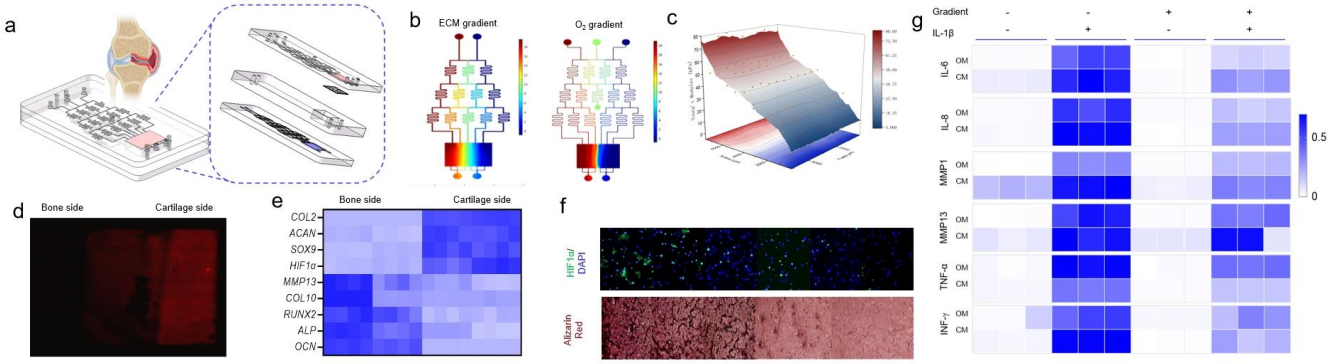

Fig. 1 Construction and characterization of OCoC and OA modeling. (a) Chip structure. (b) Computer simulations. (c) Young's modulus distribution of the scaffold. (d) Fluorescence image of hypoxia probe. (e) Heat map of PCR results. (f) HIF1α and Alizarin red staining. (g) Heat map of ELISA results.

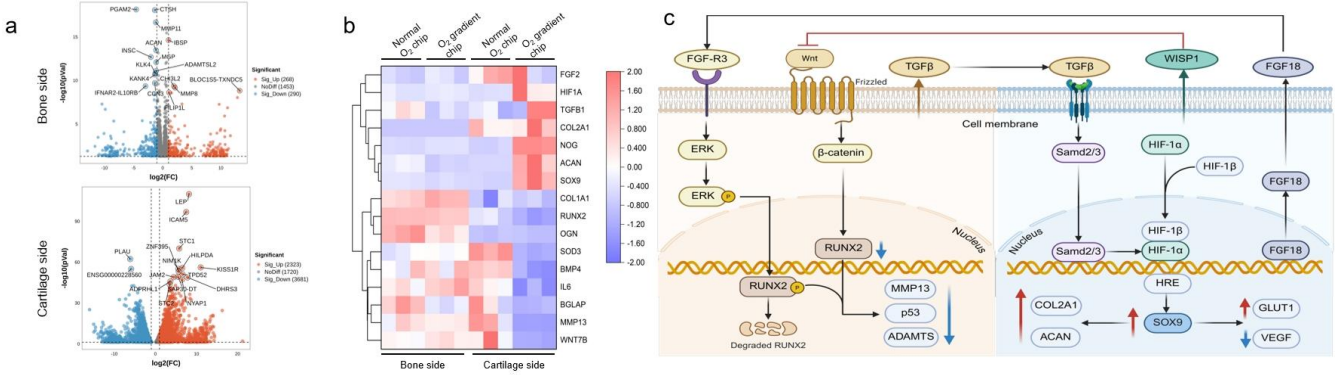

Fig. 2 The signaling pathways related to bone–cartilage crosstalk revealed in the OCoC-based OA model. (a) Volcano plots and (b) Heat map of RNA-seq results. (c) Diagram of bone–cartilage crosstalk at the OC interface as revealed by RNA-seq.