Calcium Oscillations in Primary Osteoblast Cells Influenced by Cell Orientation, Cell Spacing and Fluidic Shear Modes Base on Confined Single-cell Pattered Microfluidic Platform

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Abstract

The integration of cellular patterning methods with microfluidics provides an ideal and precisely controllable platform for quantitatively investigating the coupling reaction between force environment and multicellular systems. Here, a spatial-single cell patterning method was developed by combining micro-contact printing (μCP) and reversible microfluidic chip mounted with vacuum together. Using this platform, fluidic shear stress (FSS)-induced intracellular calcium ([Ca2+]c) signaling and spatiotemporal characteristics iwere investigated systematically, exposed to multiple spatial factors, including intercellular spacing, cell orientation, and FSS stimulation modes. Results demonstrated that closely-arranged primary osteoblasts exhibited more regular [Ca2+]c oscillations, while alignment of cell orientation with FSS direction enhanced temporal homogeneity of calcium signals. Quantitative analysis of [Ca2+]c oscillations under two mechanical modes (Steady vs. bidirectional flow, altering flow direction every 5 seconds) revealed that bidirectional flow induced more frequent and broader-range calcium oscillations compared to steady flow, alongside enhanced intercellular calcium communication. Notably, TRPV4 channel activation by 4αPDD produced calcium oscillations with similar frequency ranges, suggesting TRPV4 as a potential mediator of FSS-triggered [Ca2+]c oscillations. In TRPV4-knockout cells, the disparity in [Ca2+]c responses between bidirectional and steady flow modes became more pronounced, in clue of TRPV4's critical role in distinguishing different force modes. Furthermore, Piezo1 inhibition (GsMTx4) in TRPV4-knockout cells nearly abolished oscillatory calcium signals, yielding predominantly smooth responses under both flow conditions. These findings highlight the synergistic interplay between TRPV4 and Piezo1 in decoding external mechanical cues and calcium signaling. Our work advances the understanding of how multicellular systems interpret mechanical environments, offering some novel insights into mechanotransduction mechanisms.

Keywords

Microfluidics, Single-cell Patterning, Calcium Oscillations, Bidirectional Shear Stress, Osteoblasts, TRPV4 Channel, Piezo1