Intercellular propagation of signal induced by mechanical stimulation of a single bone marrow cell

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INTRODUCTION: Skeletal repair is influenced by mechanical loading. Mechanical loading inhibits bone resorption by osteoclasts and increases bone formation by osteoblasts, whereas unloading leads to bone loss (1). Thus osteoclasts and osteoblasts respond to mechanical stimulation in coordinated manner (2). The objective of this study was to assess how the mechanical stimulation of a single bone cell can be transmitted and perceived by other bone cells.

METHODS: Mouse bone marrow cells were cultured for 6-12 days and loaded with calcium-sensitive dye Fura-2-AM. Single cell was mechanically stimulated by gentle touch with a glass micropipette, and real-time changes in cytosolic free calcium concentration ($[Ca^{2+}]_i$) in stimulated cell and its neighbors were monitored using fluorescence microscopy (*Fig.1)*

RESULTS: Mechanical stimulation of a mononuclear, attached, well spread cell, likely osteoclast or osteoblast precursor, resulted in an increase in $[Ca^{2+}]_i$. In 11 out of 14 experiments mechanical stimulation of a single cell also led to subsequent rise in $[Ca^{2+}]_i$ in unconnected neighboring cells positioned at distance up to 140 μm away (*Fig.2*). In 3 experiments without secondary responses, change in $[Ca^{2+}]_i$ in primary cells were transient and of lower amplitude. In contrast, in 11 experiments with secondary responders, primary cells exhibited sustained calcium elevation to higher levels. Mechanical stimulation of a single cell often resulted in signal propagation to multiple secondary cells with 1 to 22 second delay from the primary response. The amplitude and duration of the responses of secondary cells was inversely proportional to delay and distance from the original cell, supporting the soluble mediator-release theory (*Fig.3* and *Fig.4*).

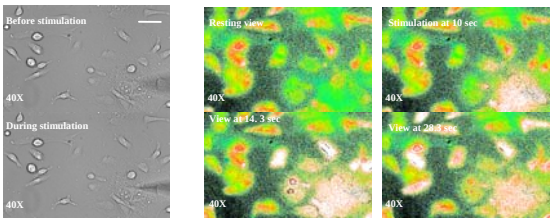

Fig.1: Bright field images showing the primary cell stimulation and snapshots of the fluorescent imaging during the experiment.

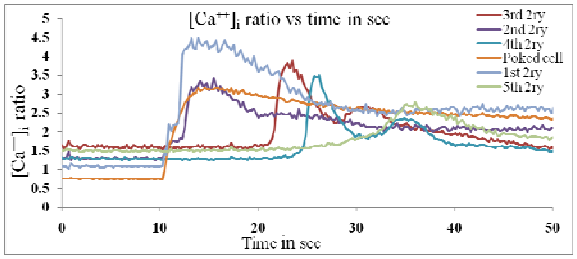

Fig.2: Mechanical stimulation produced secondary responses in unconnected neighbouring cells positioned at distance up to 140 μm away.

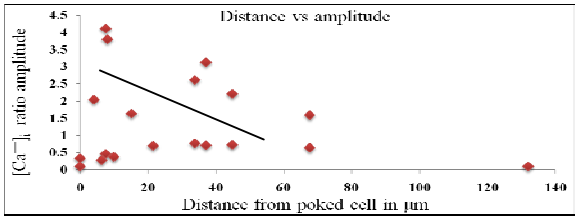

Fig.3: The more distant the secondary responder, the lower its response-amplitude

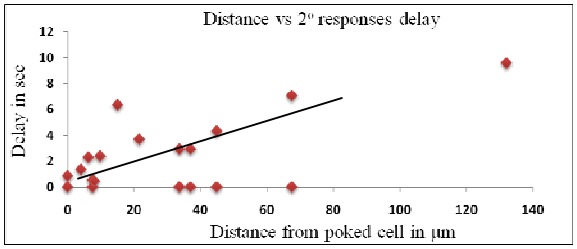

Fig.4: The more distant the secondary responder, the longer its response-delay

DISCUSSION & CONCLUSIONS: we have found that mechanical stimulation of a single bone marrow cell results in a release of a soluble mediator which transmits the signal to unconnected neighboring cells. This phenomenon may be important in coordination of the responses of different cells to mechanical stimulation.

REFERENCES: [1] R. L. Duncan, C. H. Turner (1995), *Mechanotransduction and the Functional Response of Bone to Mechanical Strain*, Calcif Tissue Int. 57:344-358. [2]Abdelilah Arredouani (2004), *Diversification of Function and Pharmacology in Intracellular Calcium Signaling*, Cellscience, Vol. 1 No. 1.

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