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Calcium Response and Transfer in Bone Cell Network with or without Gap Junctions under Mechanical Stimulation

Bo Huo, Man Hu, Ping Li

Summary

It has been widely accepted that movement of human body causes the fluid flow through pores or channels inside bone and subsequently on osteoblasts on the surface of trabeculae and osteocytes inside lacunae. The mechanism of calcium response in a bone cell and calcium transfer between bone cells is critical in understanding the communication between bone cells and calcium deposition on bone matrix. Our previous works have demonstrated that when micropatterned osteoblastic cell network with gap junctions was exposed to fluid flow, extracellular ATP diffusion following the activation of calcium response in neighboring cells plays more important roles in calcium transfer among cells. In this study, we are going to further clarify the mechanotransduction pathway of calcium transferring by using osteoblast cell network without physical connections and compare the spatiotemperal characteristics of calcium response with micropatterned osteoblasts with physical connections.

Osteoblast-like cell line of MC3T3-E1 was seeded on the micropatterned islands and there was no physical connection between cells. A parallel plate flow chamber was used to apply wall shear stress of 20 dyne/(cm*cm) on cells. At 24 hours after seeding, cells were incubated with 5 mM Fluo-4 AM, the indicator of intracellular calcium, for 2 hours. Fluorescent intensity of cytosolic calcium was recorded for 1 minute as baseline following the stimulation of fluid flow for 9 minutes. Those spatiotemporal characteristics parameters were measured and statistical analysis between different experimental groups was given. Experimental results showed that when there were no physical connections between osteoblasts, cells responded at about 120 s to first responsive peak, which is twice longer than that in osteoblast network with gap junction. When extracellular or intracellular calcium ions were removed or in case that ATP binding with purinergic receptor was inhibited, most of cells without gap junction didn't have responses to fluid flow, which is different with the cell network with gap junction. This study indicates that gap junctions between osteoblasts significantly influence intercellular calcium transferring.