

Fluid flow activation of gap junctional communication and hemichannel activity in bone cells

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Introduction

The mechanism by which bone cell networks perceive, integrate and respond to their biophysical environment is not known. We have proposed that gap junctional intercellular communication (GJIC) and release of nucleotides, specifically adenosine triphosphate (ATP), from osteocytic or osteoblastic cells are both essential to maximize bone cell response to the physical environment. Our central hypothesis is that biophysical signals, such as fluid flow, stimulate osteoblast proliferation and differentiation via a mechanism involving mobilization of cytosolic Ca^{2+} , activation of GJIC and release of ATP through gap junction (GJ) hemichannels.

Methods

We utilized a novel co-culture fluid flow apparatus to examine whether osteocytic cells exposed to mechanical signals communicate proliferation, differentiation and osteogenic signals to osteoblastic cells, a dogma of bone cell biology with surprisingly little experimental support. We also examined the role of GJ hemichannels in mechanotransduction on fluid flow activation of osteoblasts and the role of fluid flow in inducing hMSC differentiation.

Results and Discussion

Osteocytic MLO-Y4 and osteoblastic hFOB 1.19 cells were cultured on opposite sides of perforated membranes (tracer studies confirmed a migration rate through the membrane of less than 2%). This system enabled us to apply physiological levels of fluid shear to MLO-Y4 cells while permitting them to be in direct contact with hFOB 1.19 cells that are not themselves exposed to flow induced shear stress. Dye transfer analysis with calcein-AM showed that MLO-Y4 cells are coupled via gap junctions to hFOB 1.19 cells cultured on the opposite side of the membrane. GJIC between MLO-Y4 and hFOB 1.19 cells was completely blocked by the application 30 μ M α GA to the culture system.

MLO-Y4 cells were exposed to flow sufficient to induce a shear stress of 5 dynes/cm² for 1 hour and post incubated the co-cultures for 2 hours at 37^oC. Under these conditions a highly significant increase in alkaline phosphatase activity was detected in the hFOB 1.19 cells in contact with flowed MLO-Y4, compared to un-flowed control co-cultures (P<0.01). Interestingly, when hFOB1.19 were exposed either to direct flow or to conditioned media from flowed MLO-Y4 they did not display an increased alkaline phosphatase activity. We also demonstrated that MLO-Y4 cells exposed to fluid flow display Lucifer yellow dye uptake indicative of activated GJ hemichannels. Interestingly, fluid flow did not activate hemichannels in osteoblastic MC3T3-E1 cells. Fluid flow activated hemichannel activity and ATP release were significantly attenuated in cells transfected with Cx43 siRNA suggesting that flow activated hemichannels composed of Cx43 mediate ATP release. We also found that oscillatory fluid flow induced a rapid, flow rate-dependent increase in $[Ca^{2+}]_i$, that triggered a 116% increase in proliferation of human mesenchymal stem cells (hMSC) and resulted in increases in steady state levels of osteocalcin, type 1 collagen and osteopontin mRNA. Exposure to ATP also resulted in an increase in hMSC proliferation and apyrase inhibited flow induced proliferation. These results suggest that fluid flow stimulates hMSC proliferation and differentiation, perhaps through release of ATP.