Introduction: Bone is a unique material capable of modifying its material properties to meet functional demands. Examples of this process include: 1. patients subjected to prolonged bed rest who suffer disuse osteopenia, 2. immobilization of a limb resulting in loss of range of motion and decreased bone mineral density, and 3. time spent in microgravity during space flight. We hypothesized that cells in bone must coordinate their activities in order to respond to mechanical load and do so by communicating mechanical load signals via gap junctions. Gap junctions are ion channels that allow cells to pass signaling molecules between cells such as IP3 or Ca2+ ions. We utilized osteoblast-like MC3T3-E1 cells to test responses to mechanical load in vitro in two ways: 1. to assess the ability of cells to respond to cyclic load by expressing connexin 43 protein and 2. to test cell response to two different equibiaxial cyclic substrate strain rates by assessing connexin 43 mRNA expression.

Methods: MC3T3-E1 cells were cultured on Type I collagen coated plastic culture plates in DMEM-H with 10% FBS and antibiotics. BEPE5 pH 7.2, and 0.5 mM ascorbate (GM, growth medium). A Flexercell Strain Unit FX3000 with loading stations was used to provide equibiaxial strain control to cells grown on the rubber bottom culture plates. Cells were plated at 25k cells/cm2 in collagen bonded, rubber bottom BioFlex plates in GM, allowed to attach for 24h, then subjected to 8 h of peak strains of 10k to 40 k strain with strain rates of 10 or 40 k strain/sec (S1 and S2 respectively, equibiaxial strain) at frequencies of 0.5 and 2 Hz and 16 h rest (Figure 1). On days 1 and 2, cells were collected to assess protein semi-quantitation by Western blot and reaction with anti-connexin 43 antibody and a secondary antibody in an ECL reaction. Control or MC3T3-E1 cells were subjected to two different strain regimens (S1 and S2), washed free of medium, and total RNA isolated from each 6 well plate. For Northern analyses, 10 µg of total RNA were separated on 1% agarose/formaldehyde gels, blotted, hybridized with the 32P-dCTP labeled cx43 cDNA, washed with low stringency washing, and the blot exposed to autoradiographic film for 24, 48 and 72h. The 43kd Western bands and 3.3 kb connexin 43 mRNA bands were scanned, digitized and pixel density quantitated by image analysis. All data were analyzed by ANOVA and a Fisher's exact test.

Results: Data in Figure 2 show that connexin 43 protein was increased in mechanically stimulated MC3T3-E1 ostoblast-like cells by 7.2 fold and 9.8 fold respectively on days 1 and 2 post-load (p=0.001 each). Total connexin 43 protein was quantitated by adding the density of bands for both phosphorylated and nonphosphorylated forms (cx43 and cx43-p). Data in Figure 3 show that cells subjected to a lower strain rate in the first day after plating cells had a stimulation in expression of cx43 mRNA by Northern blot analysis. A load regimen with the same peak strain of 10k strain and same duty cycles (3600) but a 4 fold greater strain rate (2 Hz) compared with 0.5 Hz did not increase cx43 mRNA expression. However, by day 3 of a daily treatment of both low and high strain regimens, cells treated with the low strain rate did not demonstrate as great an amount of cx43 mRNA as did cells treated with the higher strain rate (Figure 3).

Discussion: Bone cells respond to cyclic strain in vitro, ex vivo and in vivo by signaling, dividing and increasing bone mineralization (2,3,4). However, it is unclear which form of mechanical energy acts as a stimulus to bone formation, ie, fluid shear stress, mechanical strain or the rate of change of strain. Data from in vivo studies performed on rat tibias indicate that dynamic rather than static loading is required for new bone formation and that bone formation increases linearly with increasing strain rate. However, the effect of strain rate on osteoblast response has not been reported, particularly with respect to expression of the gap junction protein connexin 43. Cyclic strain in vitro stimulated cx43 protein at days 1 and 2 post-strain in newly plated cells. Moreover, expression of connexin 43 mRNA with strain rate and time with two different strain regimens. We interpret these results to indicate a kinetic effect on connexin mRNA expression. Cells subjected to a low strain rate stimulate messenger response first then the effect diminishes whereas, the higher rate stimulates expression later as the cells subjected to the low rate reduce expression. Protein levels remain high on days 1 and 2, possibly by message stabilization. Therefore, mechanical load may act in two ways: one to stimulate cx43 mRNA expression likely as a result of maximum activation of the connexin 43 promoter by a load response gene element such as a CRE (cAMP response element) or AP1 site which are abundant in the promoter, and the second by message stabilization so that cx43 protein can be translated efficiently.