Osteocytes exhibit multiple calcium oscillations in response to mechanical loading

+[1]Al-Dujaili, SA; [1]Liu, C; [1,2]Guenther, A; [1,2]You, L
+[1]Institute of Biomaterials & Biomedical Engineering, [2]Mechanical & Industrial Engineering, University of Toronto, Toronto, ON
s.al.dujaili@utoronto.ca

Introduction: Bone is known to adapt to its mechanical environment by remodeling. External mechanical forces on bone are thought to induce physical disturbances in the lacuno-canalicular bone network that include vibration, hydraulic pressure and interstitial fluid flow. Osteocytes in the lacuno-canalicular network are believed to sense these physical stimuli and translate them into biochemical signals that regulate bone remodeling.[1] One of the early responses of osteocytes to mechanical stimuli is calcium signaling,[2] in which a transient increase in intracellular calcium concentration shortly follows the onset of mechanical stimulation.

The magnitude, length and frequency of intracellular calcium oscillations have been implicated in regulating gene expression, cell proliferation, differentiation, and apoptosis.[3] While it has been shown that osteoblasts and smooth muscle cells exhibit multiple calcium oscillations in response to stimulation, little is known about the behavior of osteocytes. Multiple calcium oscillations in osteoblasts have been suggested to be modulated by cell separation distance, indicating that gap junctions may play a role in calcium propagation and/or interactions between different cells, such as signaling between osteoblasts on the bone surface and osteocyte networks.[4] Furthermore, the magnitude of the initial calcium oscillation relative to subsequent responses has been shown to differ in osteoblasts under different forms of mechanical loading.[5,6] In this study, we hypothesize that calcium signaling response in osteocytes is mechanical loading pattern dependent and is regulated by intercellular-spacing.

Methods:

Cell culture: MLO-Y4 osteocyte-like cells (gift of Dr. Lynda Bonewald, UMKC) were maintained in alpha-MEM with 2.5% FBS, 2.5% CS, and 1% PS (all Invitrogen). Cells were seeded onto rat tail collagen I-coated glass slides, and allowed to reach 80% confluency prior to conducting oscillatory fluid flow or cyclic hydraulic pressure experiments.

Calcium imaging: Cells were rinsed twice with PBS prior to incubating with 10 μM FURA-2 AM dye (Inivtrogen) in working medium (phenol red-free alpha-MEM with 1% FBS, 1% CS, 1% PS) for 1 hour at room temperature. Cells were then washed twice with PBS and loaded onto flow or pressure chambers. Intracellular calcium response was measured as the 340 nm/380 nm ratio of fluorescence intensity.

Cyclic hydraulic pressure (CHP): Cells stained with FURA-2 AM were loaded onto pressure chambers connected to a syringe pump that delivers cyclic hydraulic pressure with 0.5 Hz triangular waveform at 68 kPa.[7] A baseline level was measured for 3 minutes, followed by 3 minutes of CHP and 3 minutes post-CHP. Responsive cells were defined as having a minimum two-fold increase in the 340 nm/380 nm ratio over the maximum baseline oscillation.

Oscillatory fluid flow (OFF): After incubation with FURA-2, cells were loaded onto parallel plate flow chambers,[2] which were connected to a linear actuator calibrated to deliver oscillatory fluid flow at 3 Pa and 1 Hz. Cells were imaged for 3 minutes of no flow prior to onset of OFF (i.e. baseline), 10 minutes OFF, and 3 minutes no flow. Cells responding to OFF were determined as those with a minimum four-fold increase in the 340 nm/380 nm ratio over the maximum baseline oscillation.

Results:

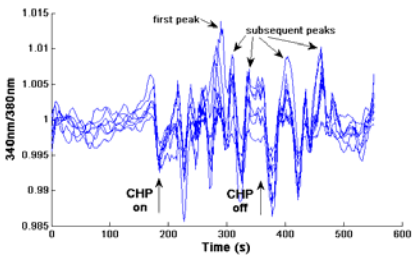

Figure 1 Intracellular Ca^{2+} response of MLO-Y4 cells to CHP. Cells exhibit multiple peaks in the 340 nm/380 nm ratio at the onset of CHP, which continue to take place up to 2 minutes after the end of CHP.

CHP induces calcium mobilization in 20.6% of MLO-Y4 cells, with 14.7% of cells having multiple responses (minimum 2-fold increase over the maximum baseline 340 nm/380 nm ratio).

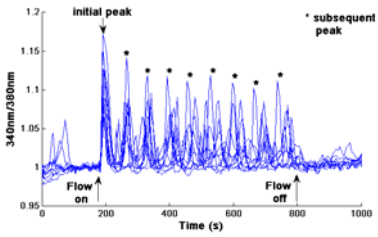

Figure 2 OFF-induced calcium response of MLO-Y4 cells. Multiple 340 nm /380 nm peaks (indicated by asterisk, *) are observed during the OFF period, but stop at the end of OFF.

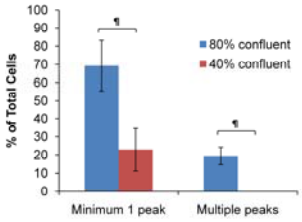

Figure 3 Reduced cell density induces significantly lower percentage of cells responding to OFF with at least one 340 nm /380 nm calcium peak. Multiple calcium peaks were not observed in non-confluent cells. Means were tested using ANOVA and Tukey post-hoc analyses, $\alpha = 0.05$ (¶ $p < 0.05$, $n = 3$).

The onset of OFF induced a large initial calcium oscillation, followed by several oscillations that immediately ceased at the end of the OFF period (Fig. 2). 69.3% of cells exposed to OFF had greater than four-fold increase in 340 nm/380 nm over the maximum baseline oscillation; 19.5% of cells have subsequent oscillations (Fig. 3). However, in low cell densities (40% confluency), the percentage of responsive cells was decreased by a factor of 3, and there were no subsequent oscillations.

Discussion:

In this study we investigated the effect of two different mechanical loading patterns on MLO-Y4 osteocyte-like cell intracellular calcium response. We found that cyclic hydraulic pressure induced a much smaller response in MLO-Y4 cells than oscillatory fluid flow. Furthermore, the number of cells with multiple calcium oscillations was also dependent on the mechanical loading pattern, where OFF induced the most number of cells with multiple responses. It is interesting to note that the calcium response in cells exposed to CHP continued for several minutes after the end of the loading period. This suggests that either a) CHP does not deplete the intracellular calcium stores as fast as OFF, or b) cells have a mechanical loading "memory" in CHP conditions, which has been postulated to modulate signaling between osteocytic networks and osteoblasts on the bone surface.[4] While our 3 Pa OFF is at the upper limit of physiological loading, 68 kPa is in the lower range of physiological CHP; additional loading magnitudes need to be explored to further characterize osteocyte response to CHP and OFF.

One limitation of our in vitro cell model is the lack of cell processes, which contain gap junctions important for sensing mechanical stimuli.[8] Here, we manipulated cell density to model the presence or absence of cell-cell contact during calcium signaling. Our data shows that cell density plays an important role in the calcium signaling response of osteocytes. Not only is the percentage of responding cells significantly reduced in low density cells, but subsequent calcium oscillations are also inhibited. This suggests that osteocytic intracellular calcium mobilization and, in particular, multiple calcium responses are dependent on intercellular spacing. This agrees with in vitro work showing decreased calcium wave propagation with increasing separation distance between osteoblasts.[4] Further work is needed to determine the role of gap junctions in regulating the effect of intercellular spacing on the magnitude and frequency of calcium response.

Significance: This is the first study to examine the role of different mechanical and spatial conditions on osteocyte calcium response. The calcium ion is an important signaling molecule involved in bone remodeling. Understanding the calcium signaling response of osteocytes to mechanical stimuli may lead to optimizing mechanical loading (i.e. exercise) as a treatment to osteoporosis and other bone diseases.

Acknowledgments: This work is supported by NSERC and CFI funds.

References: 1) Bonewald. JBMR, 2011. 2) Jacobs et al. J Biomech, 1997. 3) Thomas et al. FASEB, 1996. 4) Guo et al. MCB, 2006. 5) Donahue et al, J Biomech, 2003. 6) Huo et al. Phil Transac A, 2010. 7) Liu et al. Bone, 2010. 8) Jiang et al. Front Biosci, 2007.