Osteocytic Network Is More Responsive in Calcium Signaling Than Osteoblastic Network under Fluid Flow
+*Lu, X.L.; **Hu, B; *Baik, A D ; *Chang, V; *Guo, X E
+*Columbia University, New York, NY; **Institute of Mechanics, Chinese Academy of Sciences, Beijing, P. R. China
xf79@columbia.edu

INTRODUCTION:
Osteocytes are believed to comprise a sensory network in bone that monitors in vivo mechanical loading and triggers appropriate adaptive responses. A recent study using a targeted ablation of osteocytes in a transgenic mouse model demonstrated that the loss of osteocytes alone can induce osteoporosis, indicating the critical role of osteocytes in detecting mechanical signals and maintaining skeleton integrity [1]. It is also well recognized that osteoblasts, the cells responsible for bone formation, can directly sense and respond to mechanical stimulation (e.g., fluid flow). However, few studies have compared the sensitivity of osteocytes and osteoblasts to mechanical load [2]. In the present study, two types of cell networks were constructed in vitro with osteocyte-like and osteoblast-like cells, respectively, using microcontact printing and self assembled monolayer (SAM) technologies. The calcium responses of the cell networks to fluid flow were recorded, quantitatively analyzed, and compared.

METHODS:
A polydimethylsiloxane (PDMS) stamp with a designed grid micropattern was produced by curing a PDMS elastomeric mixture on a photore sist master replicated from a chromium mask. After incubating with an adhesive SAM, octadecanethiol, the stamp was pressed onto the gold coated glass slides. The slide was then immersed in a non-adhesive SAM solution (HS-C11-EG3, Prochimia) and further incubated with fibronectin. Osteoblast-like MC3T3-E1 cells or osteocyte-like MLO-Y4 cells were then seeded on these patterned slides and cultured for 24 hours before experiments [3]. A typical patterned cell network is shown in Fig. 1. All the cells were connected with four neighboring cells through functional intercellular gap junctions. To monitor [Ca2+], the cells were loaded with calcium-indicating fluorescence dye. During the fluid flow experiment, the slide was mounted in a parallel flow chamber and connected to a magnetic gear pump to generate a steady fluid flow with a constant 40 dynes/cm² shear stress on the cell surface. The [Ca2+], responses of the bone cell network were recorded by a high speed CCD camera for a period of 10 minutes: one minute for baseline and 9 minutes after the onset of flow. To quantitatively analyze the temporal characteristics of the recorded [Ca2+], responses, a set of parameters were defined and illustrated in Fig. 2, including the total number of responsive calcium peaks, the time to reach the first calcium peak, the relaxation time, and the time in-between successive calcium peaks. The results from the two different cell types were compared with a two-sample t test.

RESULTS:
A set of typical [Ca2+] response curves from MC3T3-E1 and MLO-Y4 cells are shown in Fig. 3. Both cells display multiple calcium spikes during the 9-minute shear loading period. The MC3T3-E1 cells released a strong [Ca2+] spike at the onset of flow which was later followed by a few weaker spikes. MLO-Y4 cells, however, tended to demonstrate more spikes without a significant decrease in the [Ca2+] spike magnitude. This suggests that not only can the MLO-Y4 cells refill their calcium store faster than MC3T3-E1 cells can, these two types of cell may have different mechanisms in releasing multiple [Ca2+] spikes. The mean value of the number of responsive peaks of MLO-Y4 cells is 6.7±3.8 (mean±sd), which is significantly higher than that of MC3T3-E1 cells (2.9±1.8, p<0.05) (Fig. 4A). Moreover, 97% MLO-Y4 cells can reach the first [Ca2+] peak in less than 20 seconds (16±4 s), significantly shorter (p<0.001) than the MC3T3-E1 cells' time (36±26 s) (Fig. 4B). This implies that either the extracellular Ca2+ can be pumped into MLO-Y4 cells faster, or the intracellular Ca2+ store in MLO-Y4 cells can release Ca2+ in a shorter time, or both. It also takes MLO-Y4 cells less time to lower its high [Ca2+] concentration (Fig. 4C; MLO-Y4: 23±13 s, MC3T3: 44±70 s, p<0.05). No significant difference is detected for parameter t1, time between first and second peaks, in the two groups. A negative exponential correlation between number of responsive peaks and t1 is shown in Fig. 5 for MLO-Y4 cells (R² = 0.44).

SUMMARY:
In conclusion, under the same mechanical stimulation, MLO-Y4 cells can release [Ca2+] spikes at a much higher frequency than MC3T3-E1 cells can. It also takes a much shorter time for the MLO-Y4 cells to release a [Ca2+] spike after the onset of flow and to recover the intracellular calcium store. These results may imply that osteocytes, as the major mechanical sensor in bone, are more sensitive and active under mechanical stimulations than the osteoblasts are.

Figure 1 (L): A fluorescence image of a patterned MLO-Y4 cell network in pseudo-color; Figure 2: A typical calcium response of a bone cell and the definitions of temporal parameters employed in the present study.

Figure 3 (L): Two typical calcium response curves from MC3T3-E1 and MLO-Y4 cells; Figure 5 (R): For MLO-Y4 cells, the number of responsive [Ca2+], peaks is correlated with the time between successive peaks.

Figure 4: Comparison of temporal parameters in [Ca2+], responses from MC3T3-E1 and MLO-Y4 cells: (A) total responsive times during steady fluid flow stimulation; (B) time to reach the 1st [Ca2+], peak after the onset of flow; (C) time to 50% relaxation after the 1st peak; (D) time between 1st and 2nd peaks.

REFERENCE: