INTRODUCTION

The ability of bone tissue to remodel its mass and geometry in response to varying mechanical stimuli has been a long recognized phenomenon, but the cellular mechanisms behind this phenomenon are poorly understood. The biological events involved with bone adaptation are a current focus of intense research. Previous studies have indicated that bone formation, mineral content, and bone matrix protein production are reduced when forces on the body during space flight are reduced. Knowledge of these cellular mechanisms will lend a good deal of knowledge to the understanding of bone adaptation and certain bone disorders such as osteoporosis.

Mechanotransduction is the cellular event where mechanical stimuli are converted into biochemical signals. Mechanotransduction is believed to regulate bone adaptation to mechanical loading. With an applied load, it is believed that fluid flow in the porous spaces of bone may help adaptation by providing the osteoblastic cells with physical stimulation and information on the overall stresses acting on the bone as a whole, while enhancing molecular transport.

One of the earliest events in mechanotransduction is intracellular calcium signaling. In vitro, bone cells respond to fluid flow with oscillations in intracellular calcium concentration ([Ca\(^{2+}\)]). The oscillation magnitude, duration, and frequency of [Ca\(^{2+}\)] oscillations in many cell types have been implicated in the regulation of downstream biological events such as gene expression, differentiation, and apoptosis. The characteristics of [Ca\(^{2+}\)] oscillations, such as the oscillation magnitude, duration, and area under the curve define a calcium “fingerprint” for individual cells. It has been suggested that calcium fingerprints also play an important role in determining downstream events.

We hypothesized that the oscillation magnitude, duration, and area under the oscillation curve for fluid flow induced [Ca\(^{2+}\)] oscillations in individual bone cells were equivalent during multiple bouts of oscillating fluid-flow separated by rest periods. In other words, we hypothesized that mechanically stimulated bone cells have calcium fingerprints.

METHODS

Bone Cells Subperiosteal osteoblastic cells were isolated from the humeri, tibiae, and femora of male Fisher 344 rats using established methods. The soft tissues were stripped from the bones, and sequential collagenase digestions at 37°C isolated the cells. Cells from the second digestion were collected by centrifugation and grown to confluency in DMEM, 20% FBS, and 1% penicillin/streptomycin. Cells were plated on quartz microscope slides at concentrations that reached 70% confluency on the day of experimentation. Cells were incubated at 37°C with 10 µM of the fluorescent dye Fura-2 AM for 30 minutes prior to fluid flow.

Fluid Flow System Following incubation with Fura-2 the slides were mounted into a parallel plate flow chamber on the stage of a fluorescent microscope. A materials testing machine drove the fluid through the chamber with an oscillating profile. DMEM and 2% FBS constituted the flow media. Following a one-minute baseline period, cells were exposed to 2 minutes of oscillating fluid flow, a thirty-minute rest period, a second baseline period, and a second 2-minute fluid flow period. The fluid flow produced a peak shear stress of 20 dynes/cm\(^2\) at a frequency of 2 Hz. Real-time [Ca\(^{2+}\)], for individual cells was calculated using radiometric dye methodology and image analysis software (Metaflour, West Chester, PA).

Data Analysis The [Ca\(^{2+}\)], oscillations of individual cells were analyzed with engineering spreadsheet software (DADiSP, Newton,
MA). The oscillation magnitude, duration, and area under the curve of the [Ca\(^{2+}\)], versus time profile were calculated for each cell for both flow periods. The determining factor for the oscillation behavior was whether or not the intracellular calcium concentration exceeded the baseline value by 25 percent. Linear regressions were used to determine the correlation coefficients for these parameters between the two flow periods, which allowed for evaluation of the similarities in behavior between the two oscillation periods.

**RESULTS**

Of the 50 cells that were analyzed, 42 displayed [Ca\(^{2+}\)], oscillations during the first bout of flow. The majority of the cells (41 out of 42) that responded to the first bout of flow responded to the second bout of flow with the same size and shape [Ca\(^{2+}\)], versus time profile (Figure 1). Both flow periods produced immediate [Ca\(^{2+}\)], oscillations with durations of approximately 85 seconds, and oscillation magnitudes of approximately 150nM; the areas under the oscillation curve were approximately 6700nM*seconds (Table 1). There were no significant differences, between the first and second oscillations, in the oscillation magnitude (p<0.0001, R\(^2\)=0.717), duration (p<0.0001, R\(^2\)=0.948), and area under the oscillation curve (p<0.0001, R\(^2\)=0.947).

<table>
<thead>
<tr>
<th>Flow Period 1</th>
<th>Oscillation Magnitude (nM)</th>
<th>Duration (seconds)</th>
<th>Area Under Curve (nM*s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Period 2</td>
<td>150 ± 41</td>
<td>85 ± 35</td>
<td>6852 ± 3052</td>
</tr>
</tbody>
</table>

Table 1: Means and standard deviations (n=41) of the oscillation magnitude, duration, and area under the curve of the [Ca\(^{2+}\)], oscillations for the two fluid flow periods.

Our findings may have important implications for the role of [Ca\(^{2+}\)], signaling in bone cell mechanotransduction and bone adaptation. In vivo, a rest period inserted between consecutive loading bouts enhances bone formation. Previously, we showed that rest periods were required for multiple, fluid-flow induced calcium oscillations in bone cells. Understanding the role of [Ca\(^{2+}\)], fingerprints in bone cell mechanotransduction may help us understand in vivo bone adaptation mechanisms. For example, understanding the variability of individual calcium response patterns could lead to a better understanding of downstream events such as bone cell apoptosis, differentiation, and gene expression. It may also increase our understanding of how rest periods enhance mechanically induced bone formation in vivo.

**REFERENCES**


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