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Differential effect of steady versus oscillating flow on bone cells

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Abstract

Loading induced fluid flow has recently been proposed as an important biophysical signal in bone mechanotransduction. Fluid flow resulting from activities which load the skeleton such as standing, locomotion, or postural muscle activity are predicted to be dynamic in nature and include a relatively small static component. However, in vitro fluid flow experiments with bone cells to date have been conducted using steady or pulsing flow profiles only. In this study we exposed osteoblast-like hFOB 1.19 cells (immortalized human fetal osteoblasts) to precisely controlled dynamic fluid flow profiles of saline supplemented with 2% fetal bovine serum while monitoring intracellular calcium concentration with the fluorescent dye fura-2. Applied flows included steady flow resulting in a wall shear stress of 2 N m^{-2} , oscillating flow ($\pm 2 \text{ N m}^{-2}$), and pulsing flow (0 to 2 N m^{-2}). The dynamic flows were applied with sinusoidal profiles of 0.5, 1.0, and 2.0 Hz. We found that oscillating flow was a much less potent stimulator of bone cells than either steady or pulsing flow. Furthermore, a decrease in responsiveness with increasing frequency was observed for the dynamic flows. In both cases a reduction in responsiveness coincides with a reduction in the net fluid transport of the flow profile. Thus, these findings support the hypothesis that the response of bone cells to fluid flow is dependent on chemotransport effects.

1. Introduction

Bone tissue is continuously being formed and resorbed in a highly regulated process dependent on tightly coordinated cellular activity. This bone turnover is regulated by a number of biochemical and hormonal factors and is also influenced by mechanical loading (Jones et al., 1977). This fact necessitates the existence of one or more mechanisms of biological mechanotransduction. Mechanotransduction in bone has been proposed to involve a variety of biophysical signals including streaming potentials, piezoelectric potentials, damage to the extracellular matrix, and direct transduction of matrix strain. Recently, fluid flow has been discovered to be a potent stimulator of bone cells in vitro (Hung et al., 1995; Reich et al., 1990; Reich and Frangos, 1991) and has been put forward as an important biophysical signal in mechanotransduction (Cowin et al., 1995; Weinbaum et al., 1994, 1991). Indeed, experimental evidence has suggested that fluid flow is a more potent stimulator of bone cells than substrate deformation (Owan et al., 1997). In this project we address the issue of how various flow profiles affect the responsiveness of bone cells in vitro. Specifically we consider the difference between oscillating, pulsatile, and steady flows, the effect of frequency in dynamic flow, and the role of fluid flow's chemotransport effect.

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As bone tissue is loaded in vivo, fluid in the lacunar/canalicular network experiences a heterogeneous pressurization in response to the deformation of the mineralized matrix. This leads to fluid flow along pressure gradients. When loading is removed, pressure gradients and flows are reversed. These fluid motions are dynamic and oscillatory in nature. Furthermore, although load-induced fluid flow rates have not been measured directly, the oscillatory component of the bone cell's fluid flow environment has the potential to greatly exceed the steady component of fluid flow driven by the arterial pressure head suggesting that oscillating flow may be the most appropriate flow regime for in vitro study. In addition to repetitive loading due to locomotion, oscillating fluid flow has also been suggested to result from high-frequency muscular loading associated with postural control (Weinbaum et al., 1994). The effect on bone cell metabolism of steady and pulsatile flow regimes has been studied in detail in the past (Ajubi et al., 1996; Frangos et al., 1988; Hillsley and Frangos, 1997; Hung et al., 1995; Reich et al., 1990, 1997; Reich and Frangos, 1991, 1993). Despite the fact that oscillating flow may be a better approximation of the physiologic conditions bone cells experience in vivo, the effect of oscillating flow on bone cell signal transduction has not been quantified. The study described herein represents the first investigation into the effect on bone cell signal transduction of well-defined purely oscillating fluid flow.

The fluid flow behavior of the lacunar/canalicular network has been modeled from a theoretical standpoint (Cowin et al., 1995; Weinbaum et al., 1991, 1994). Using available data regarding the physical properties of the lacunar/canalicular spaces as well as the occupying osteocytes and interconnecting processes these models have been verified in that they make accurate predictions of bulk tissue fluid permeability and streaming potentials. Interestingly, these models also predict that the magnitude of shear stress imposed on the cell is dependent on the frequency of the applied loading. However, no prior investigations have been performed to assess frequency sensitivity in the response of the cell to load induced flow. Thus, another goal of this project was to quantify any frequency dependence in bone cell mechanotransduction.

In addition to its direct effects on cells, loading induced fluid flow oscillations may be a mechanism for increasing nutrient exchange in bone (Johnson, 1984; Kufahl and Saha, 1990; Piekarski and Munro, 1977). Experimentally, physiologic levels of mechanical loading applied to bones in situ have been shown to increase the levels of fluorescent tracers accumulated in bone tissue (Knothe Tate et al., 1998) indicating that chemotransport is mechanically regulated in vivo. Thus, if fluid flow induced chemotransport can be shown to influence cell metabolism, it may have a significant role in mechanotransduction distinct from flow induced shear stress. However, experiments aimed at determining the role of chemotransport are contradictory. Allen et al. (1997) have demonstrated that primary cultures of neonatal rat calvarial osteoblasts have two distinct fluid flow response mechanisms which are activated depending on whether serum is present in the media. They found that the serum-dependent mechanism involves a pertussis toxin sensitive G-protein and the serum-independent mechanism does not. Conversely, by employing fluid media of various viscosities modified with the addition of neutral dextran, Reich et al. (1990) concluded that bone cell responsiveness depended on shear stress level, but not on fluid flow rate. Thus, the final goal of this project is to determine the role of chemotransport in bone cell mechanotransduction by controlling net fluid transport independent of shear stress level.

A wide variety of measures of cell responsiveness have been employed in investigations of bone cell's response to mechanical stimulation. These include changes in gene transcription (Sun et al., 1995), prostaglandin release (Ajubi et al., 1996; Reich and Frangos, 1991), NO production, electrophysiological behavior (Duncan and Hruska, 1994) and intracellular calcium concentration (Hung et al., 1995). In this study we have elected to quantify intracellular calcium concentration $[Ca^{2+}]_i$ as a measure of responsiveness. Intracellular

calcium is an early response second messenger that plays a role in a number of metabolic pathways, and is typically observed to increase dramatically within seconds of cell stimulation. As a second messenger, $[Ca^{2+}]_i$ transduces extracellular changes (i.e. first messengers) to the cell interior and potentially to the genome and is important in regulation of cellular metabolism. Furthermore, using fluorescent imaging techniques described below it is possible to quantify $[Ca^{2+}]_i$ changes in response to fluid flow in vitro within individual cells in real time.

In summary, the first goal of this study was to contrast the effects on bone cell mechanotransduction, in terms of intracellular calcium signalling, of fluid flow applied in three distinct precisely controlled fluid velocity regimes, steady, pulsatile, and oscillating. Secondly, we studied the effect of frequency on bone cell response in the time varying pulsatile and oscillating flow conditions. This approach allowed us to distinguish the effects of flow induced shear from the effects of fluid transport by applying flows which do or do not include a net transport of media.

2. Methods

2.1. Cell culture

Human fetal osteoblastic cells (hFOB 1.19) (Harris et al., 1995) were cultured in Dulbecco's Modified Eagle Medium with nutrient mixture F-12 (D-MEM/F-12, Gibco, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin. They were subcultured onto quartz microscope slides and grown to 80% confluence.

2.2. Calcium imaging

Intracellular calcium ion concentration ($[Ca^{2+}]_i$) was quantified with the fluorescent dye fura-2. Fura-2 exhibits a shift in absorption when bound to calcium such that emission intensity when illuminated at a 340 nm wavelength increases with calcium concentration, and decreases with calcium concentration when illuminated at 380 nm. Calcium concentration can, therefore, be computed from the ratio of the two emission intensities independent of dye concentration and illumination intensity. The dye is supplied in a cell permeant form (fura-2-AM) which diffuses across the cell membrane. Intracellular esterases convert the dye to an impermeant form (fura-2) localizing it to the cell interior and allowing the extracellular dye to be removed.

Cells were loaded with 1 μ M fura-2-AM for 30 min prior to each experiment and placed into the parallel plate flow chamber. All experiments were performed at room temperature. The chamber was then filled with perfusate buffer (Tyrode's solution with 2% FBS) and placed on the stage of a Nikon Diaphot inverted microscope equipped for epifluorescence and computer image acquisition. Cell ensembles were illuminated at wavelengths of 340 and 380 nm and emitted light passed through a 510 nm interference filter and detected with an ICCD camera. Images were recorded at a rate of one every 2.5 s and analyzed using image analysis software (Metafluor; Universal Imaging, West Chester, PA) operating on a DECxL 590 microcomputer (Digital Equipment Corporation, Maynard, MA). Calibration ratios were obtained using fura-2 in buffered calcium standards supplied by the manufacturer. Baseline $[Ca^{2+}]_i$ was sampled for 1 min followed by a 3 min flow period. At the conclusion of each experiment, the recorded images were analyzed by playing them back with the image analysis software. Each cell was circled individually and the software computed the calibrated $[Ca^{2+}]_i$ value for each cell at each point in time. This resulted in a $[Ca^{2+}]_i$ versus time trace for each cell circled.

2.3. Flow exposure device

The parallel plate flow chamber has enjoyed widespread success in in vitro studies of cellular response to fluid flow. The design we employed is modified from Frangos et al. (1985) to accept the quartz glass microscope slides required for the fluorescent imaging technique. The chamber is made up of a polycarbonate manifold, a rubber gasket, and a microscope slide held together by vacuum pressure (Fig. 1). The resulting rectangular fluid volume measures $38 \text{ mm} \times 10 \text{ mm} \times 0.28 \text{ mm}$. The Reynolds number for this configuration at the maximum flow rate employed in this study (18 ml min^{-1}) can be computed at 54 (White, 1994). In a parallel plate configuration (called Poiseuille flow in the fluid mechanics literature), transition to the turbulent flow occurs at a Reynolds number between 1000 and 8000 (Panton, 1996). Therefore, the flow rates considered in this study are well within the laminar flow regime (Allen et al., 1997; Hillsley and Frangos, 1997). The parallel plate flow velocity profile is presented in Fig. 2. Due to the simple flow profile a closed form equation relates flow rate to shear stress at the cell layer (Hung et al., 1995).

$$\tau = 6\mu Q/bh^2$$

where τ is the shear stress, μ is the viscosity, Q is the flow rate, b is the flow channel width, and h is the flow channel height.

A streamwise distance known as the “entrance length” is required for the channel flow to become fully developed laminar flow (Fox and McDonald, 1985). A conservative estimate of the entrance length for two-dimensional flow is $0.06 \text{ Re } h$. Furthermore, the entrance length also provides a conservative upper bound on the lateral distance of boundary effects associated with the lateral boundaries of the chamber. In our configuration the entrance length is 0.90 mm . Therefore, no measurements were taken within 1 mm of the chamber boundary to ensure that cells under observation experienced purely laminar flow.

Inlet and outlet slits at either end of the manifold provide a means of providing fluid flow. An inline pressure transducer was attached to the flow chamber inlet. The static component of flow was provided with a Harvard syringe pump (Harvard Apparatus, South-natick, MA). The dynamic component of flow was provided by a 1 cm^3 syringe mounted in a servohydraulic loading machine (Interlaken model 3350, Eden Prairie, MN). Arbitrary computer generated displacement commands are followed by the servohydraulic actuator to within $50 \text{ }\mu\text{m}$ resulting in delivery of a flow profile accurate to within $0.83 \text{ }\mu\text{l}$ at any point in time. Thick rigid walled plastic tubing was used throughout the flow delivery system to minimize dynamic compliance. The static and dynamic components of flow were summed with a Y connector and delivered to the flow chamber. A schematic of the fluid flow circuit is given in Fig. 3. Fura-2 loaded cells were exposed to three different flow regimes, steady flow at 18 ml min^{-1} (2.0 N m^{-2}), sinusoidally varying flow with an amplitude of $\pm 18 \text{ ml min}^{-1}$ (oscillating), and the sum of a 9 ml min^{-1} steady and a 9 ml min^{-1} oscillating flow resulting in a flow rate varying from 0 ml min^{-1} to 18 ml min^{-1} (pulsing) (Fig. 4). Additionally, the dynamic flows were applied at frequencies of 0.5 , 1 , and 2 Hz . These frequencies are sufficiently low to allow for fully developed laminar flow to develop in the flow chamber. No-flow controls were obtained by following the flow protocol in all aspects without activating the flow delivery system.

2.4. Data analysis

We adapted a numerical procedure from mechanical fatigue analysis, known as Rainflow cycle counting, to identify oscillations in $[\text{Ca}^{2+}]_i$ from the time histories (Jacobs et al., in

review). In short, this technique identifies complete cycles or oscillations in the time history data by pairing increases and decreases that are not necessarily adjacent to one another. Rainflow analysis is unique in that it can identify distinct individual oscillations in the time history data even when they are superimposed upon each other. Rainflow analysis is a powerful tool for distinguishing and quantifying responses from background signal noise. In the current study we defined a response as an oscillation in $[Ca^{2+}]_i$ of 50 nM or greater.

2.5. Statistics

Each cell was classified as either responding or not responding by the above criteria. Responsiveness was characterized by the mean fraction of responding cells and the average response amplitude. Variability in the fraction of responding cells was characterized by the standard error of proportion and statistical comparisons were made on the basis of the z test statistic for proportions (Glantz, 1992). Response amplitudes were compared with ANOVA and the Newman–Keuls post-hoc test to identify differences between group means. Variability in response amplitudes was quantified by the standard error of the mean.

3. Results

Fluid pressure at the inlet was found to vary sinusoidally with time with a pressure variation of between 4 kPa and 6 kPa. Since the outlet was exposed to the atmosphere, we assumed that no pressure variation occurred at the chamber outlet.

Fig. 5 shows the time histories of $[Ca^{2+}]_i$ for a typical experiment. Generally, responding cells exhibited a dramatic transient increase in $[Ca^{2+}]_i$ shortly after the onset of flow although occasionally cells exhibited a delayed response or maintained elevated $[Ca^{2+}]_i$ over the duration of flow. Small magnitude oscillations were observed during the basal period due to random system noise (i.e. not cellular responses). The average maximum oscillation in $[Ca^{2+}]_i$ observed in the pre-flow period was 9.3 nM, and was thus significantly smaller than the 50 nM oscillation threshold adopted to distinguish responding cells from non-responding cells. For the no flow controls only a single cell was observed to spontaneously respond in the absence of flow with an amplitude of 56 nM ($n = 73$). In the case of steady flow 19.3 $\pm$ 2.2% ($\pm$ SEP) of individual cells responded to flow ($n = 218$) with an average amplitude of 84 $\pm$ 4.1 nM ($\pm$ SEM). The results for the fraction of cells demonstrating a response are summarized, for all experimental flow conditions, in Fig. 6. The corresponding average response amplitudes are presented in Fig. 7. For pulsing flow both the fraction of responding cells and the average response amplitude were dependent on frequency with 64.3 $\pm$ 4.8% of cells responding with a mean amplitude of 120 $\pm$ 7.8 nM at 0.5 Hz, 42.6 $\pm$ 5.1% responding with a mean amplitude of 102 $\pm$ 7.0 nM at 1.0 Hz, and 21.7 $\pm$ 4.0% responding with a mean amplitude of 109 $\pm$ 16.5 nM at 2.0 Hz. Like-wise, for oscillating flow 10.3 $\pm$ 1.8% of cells responded with a mean amplitude of 83 $\pm$ 6.1 nM at 0.5 Hz, 7.7 $\pm$ 2.0% responded with a mean amplitude of 81 $\pm$ 10.9 nM at 1.0 Hz, and 5.1 $\pm$ 1.9% responded with a mean amplitude of 79 $\pm$ 11.1 nM at 2.0 Hz.

Three important trends characterize these results. Firstly, at every frequency examined oscillating flow was significantly less stimulatory in terms of the fraction of cells stimulated than either pulsing or steady flow (in all cases $p < 0.01$). Secondly, the dynamic flows (oscillatory and pulsatile) were less stimulatory in terms of the fraction of responding cells with increasing frequency although this trend reached statistical significance only for pulsatile flow ($p < 0.01$). Finally, pulsatile flow was more stimulatory than steady flow in terms of the fraction of cells responding for 0.5 Hz and 1.0 Hz ($p < 0.01$) and in terms of response amplitude for 0.5 Hz only ($p < 0.01$). None of the response amplitudes were significantly different than steady flow except for pulsatile flow at 0.5 Hz ($p < 0.01$). These results satisfy the three aims of this study. Namely they (1) demonstrate a distinct difference

in the response of bone cells to oscillating flow in contrast with steady or pulsing flow, (2) demonstrate a strong frequency dependence for pulsatile flow and to a lesser extent for oscillating flow, and (3) suggest a significant role for chemotransport in bone cell fluid flow responsiveness. Each of these points will be discussed in detail below.

4. Discussion

To our knowledge this is the first study to examine the effect of oscillatory fluid flow on bone cells. This is significant because although bones are not loaded in a sinusoidal manner, they are loaded repetitively. As a result the induced fluid flow through the lacunar/canalicular network is reversed when the bone is unloaded. Thus, we feel that although the sinusoidal oscillating flow profiles utilized in this experiment are idealistic, they are more physiologic than steady or pulsatile flow profiles because the flow is reversing in nature.

These results clearly demonstrate that bone cells in vitro exhibit a significantly different response to oscillating fluid flow than to steady or pulsatile flow. In general terms oscillating flow appears to be significantly less stimulatory than steady or pulsatile flow. This suggests that the response mechanism activated by oscillating flow is different in part or in whole than that activated by steady or pulsatile flows, and is supported by the preliminary data of Allen et al. (1997) suggesting that there may be multiple cellular mechanisms involved in the fluid flow response of bone cells. The results further indicate that different mechanisms may be activated by distinct flow regimes. To the extent that oscillating flow represents a more realistic consequence of mechanical loading, many prior flow experiments may need to be reinterpreted.

A second goal of this study was to investigate the influence of frequency on the response of bone cells to dynamic flow profiles. We found a decrease in bone cell sensitivity with increasing frequency for the dynamic flows. A decrease in the innate sensitivity of bone cells in vitro with frequency is a significant finding as it may attenuate or counteract the increase in shear stress with frequency predicted by the theoretical model of Weinbaum et al. (1994). However, it is unclear at this point if the net result of these two phenomena will result in increasing or decreasing frequency dependence at the bone tissue level. Furthermore, the number of frequencies investigated in an experimental study must be restricted due to practical limitations on experimental resources relative to the infinite number of frequencies that can be studied theoretically. Thus, the frequency range addressed by the Weinbaum model far exceeds that studied in this study. Additional experiments will be required to determine if the decreasing sensitivity of bone cells with increasing frequency to oscillating flow will extend to the higher frequencies expected to occur with postural muscle activity.

The final goal of this study was to determine the role of chemotransport in the response of bone cells to fluid flow. This goal is addressed by our results in two ways. The primary evidence for the role of chemotransport is that a greater response was observed at all frequencies in cells exposed to a net fluid transport (steady and pulsing) relative to cells exposed to no net fluid transport (oscillating) despite the fact that the peak shear stress was the same for all flow rates. Secondly, in the case of dynamic flows a trend of decreasing numbers of responding cells with increasing frequency was observed, although the trend did not reach statistical significance for oscillating flow. However, the lack of a statistically significant frequency dependency in the oscillating flow case may be due to the lower overall responsiveness of the cells to oscillating flow. This inverse trend of decreasing responsiveness with increasing frequency corresponds to a decrease with frequency of the volume of fluid with which the cells come into contact, implicating a fluid transport-dependent mechanism. In the absence of flow the cells are in contact with 110 μ l of media in

the flow chamber. When flow is imposed, the cells come into contact with additional media, the volume of which depends on the frequency of oscillation. In the case of oscillating flow (with no steady component) of 18 ml/min at 0.5 Hz, 1 Hz, and 2 Hz, an additional volume of 191, 95, and 48 μ l respectively is repeatedly pushed in and out of the chamber and any laminar boundary layers destroyed by mixing at the inlet and outlet slits. Thus, the effective volume of media the cells are exposed to is reduced with increasing frequency. As a consequence, a yet to be defined biochemical factor within the media which is necessary for maximal cell response and is metabolized by cells would be depleted more rapidly at higher frequencies. Alternatively, a secreted inhibitory factor would accumulate faster with higher frequency. A superimposed steady component of flow (pulsatile) has the effect of continuously replenishing the effective volume with fresh media and dramatically increasing responsiveness. However, the decreased effective volume with increased frequency would still lead to increased chemotransport effects due to either enhanced depletion of an agonist or accumulation of an inhibitor. Thus, the decreased responsiveness of bone cells we observed with increasing frequency in oscillating as well as pulsatile flow is consistent with a chemotransport-dependent mechanism. This interpretation is also consistent with unpublished observations from our laboratory suggesting that the sensitivity of chondrocytes to fluid flow is dramatically increased with the addition of serum to the media and similar published results for bone cells (Allen et al., 1997). Thus we conclude that chemotransport of a serum factor plays an important role in the response of bone cells to fluid flow.

Several limitations should be considered when interpreting our results. One limitation is that in addition to exposing cells to variations in fluid flow our configuration necessarily exposed the cells to changes in pressure. We have measured the pressure drop across the chamber to be between 4 and 6 kPa. Much of this pressure drop may have occurred at the constrictions at the inlet and outlet slits. The remaining pressure drop would occur linearly from the inlet to the outlet. Pure cyclic hydrostatic pressures of 17.2 kPa have been shown to elicit oscillations in $[Ca^{2+}]_i$ of 126 nM (Brighton et al., 1996) and 13 kPa has been shown to effect alkaline phosphatase and TGF-beta activity, as well as collagen and actin expression (Roelofsen et al., 1995; Klein-Nulend et al., 1995) in bone cells. Furthermore, a 20 min 4 MPa pulse of hydrostatic pressure has been shown to increase bone cell adhesion (Haskin et al., 1993). Thus, although the pressure changes experienced by cells in our system are an important concern, they are three times lower than those that have been shown to effect cell metabolism. Other important considerations include our utilization of an immortalized cell line and the fact that our experiments were conducted at room temperature.

In this study we observed differences in $[Ca^{2+}]_i$ responsiveness, in terms of the fractions of responding cells, that are not paralleled by the response amplitude. This suggests the interesting possibility that the bone cell calcium response to fluid flow is an all-or-none response and that information regarding loading intensity is transduced by the number of responding cells rather than their response amplitude. This is in agreement with observations of the calcium response of bovine aortic endothelial cells (Geiger et al., 1992) and articular chondrocytes (Yellowley et al., 1997) to fluid flow.

Since quantifying the effect of flow rate on responsiveness was not one of the aims of this study, all of our experiments were conducted at one consistent physiologic peak flow rate (18 ml min⁻¹). It is possible that the character of our results may change at higher or lower flow rates. It is likely that multiple mechanosensitive mechanisms are present in bone cells and that these mechanisms are activated at different levels of stimulus. A biphasic response of bone cells has been observed with respect to fluid flow response duration (Reich and Frangos, 1993). Multiple response mechanisms would be advantageous in terms of biologic redundancy or may be associated with distinct mechanically regulated functions such as turnover and repair.

5. Conclusions

This experiment represents the first investigation of the effect of purely oscillating fluid flow on bone cells. We have demonstrated that in vitro oscillating fluid flow is far less stimulatory to bone cells than steady or pulsatile flow suggesting that mechanotransduction of oscillating flow may occur through a fundamentally different cellular mechanism than steady or pulsatile flow. This finding is important to an understanding of mechanotransduction in bone because loading-induced fluid flow is likely to be primarily oscillatory in vivo. Additionally, our results show that dynamic flows become less stimulatory with increasing frequency. Finally, we find that these observations are consistent with, and can be explained by, the hypothesis of a chemotransport-dependent response mechanism involving a factor contained in serum.

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Fig. 1. Schematic of the parallel plate flow chamber consisting of a quartz glass slide with cells attached, silastic rubber gasket, and polycarbonate manifold. The components are held together by vacuum. The inlet and outlet ports communicate with the inlet and outlet slots.

Fig. 2.

A vertically oriented streamwise cross-section through the parallel plate flow chamber depicting the laminar fluid velocity profile. Cells are grown on the quartz glass microscope slide that makes up the bottom of the flow chamber. The top surface is formed by the polycarbonate of the chamber body. Fully developed laminar flow exhibits a parabolic velocity profile with a maximum velocity in the center that drops to zero at the top and bottom surfaces.

Fig. 3.

A schematic of the fluid flow circuit. Steady flow is generated by a Harvard syringe pump (upper left). Oscillating flow is supplied with an Interlaken servohydraulic loading machine (upper right). Flow from the two devices are summed with a “Y” connector and input to the flow chamber via an inline pressure transducer. If the Harvard pump alone is activated steady flow is delivered to the chamber. If only the Interlaken is activated oscillating flow is delivered to the chamber. If both are activated pulsatile flow is delivered to the chamber. Finally, the flow chamber outlet is vented to the air with a short length of tubing.

Fig. 4. An example of two cycles of the three flow profiles analyzed in this study, steady, pulsing, and oscillating. Note that the dynamic flows (pulsing and oscillating) were applied at three different frequencies, 0.5, 1.0, and 2 Hz.

Fig. 5.
An example of the $[Ca^{2+}]_i$ response traces obtained for steady flow. Note the arrow depicts the onset of flow.

Fig. 6.

The fraction of cells responding to flow for each of the flow profiles studied. At all frequencies studied oscillating flow was less stimulatory than pulsatile or steady flow. Also note the trend of decreasing responsiveness with increasing frequency. The number of cells analyzed for each flow regime from left to right was; $n = 272, 181, 137, 98, 94, 106, 188, 73$. Bars represent standard error of a proportion.

Fig. 7.

The corresponding average response amplitudes for each flow profile in nM. Only the response amplitude of pulsing flow at 0.5 Hz was statistically significantly different from the steady-flow condition. Since only one cell responded of the no flow controls, comparisons of response amplitude were not possible with this group. The bars represent standard error of the mean.