CHAPTER 5

STOCHASTIC RESONANCE ENHANCES THE RAPID RESPONSE OF BONE CELLS TO FLUID SHEAR STRESS

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ABSTRACT

Stochastic resonance is manifest in non-linear systems, where the response to a small periodic signal is enhanced by noise. It is unknown whether bone cell mechanosensitivity is enhanced by a noisy loading environment as an alternative mechanism for an amplified response to stress. Since osteocytes are believed to be the mechanosensors in bone *par excellance*, we studied whether noise of varying intensities enhanced the mechanosensitivity of MLO-Y4 osteocytes in comparison with MC3T3-E1 osteoblasts. Nitric oxide (NO) and prostaglandin E_2 (PGE_2) production were measured as parameters for bone cell activation. Here we modeled the response of the cell culture to noisy fluid shear stress by an enhancement of the information content of the applied stress. We found that the NO response of MLO-Y4 osteocytes to a small periodic fluid shear stress was acutely enhanced by noise. MC3T3-E1 osteoblasts did not show an acutely enhanced NO response to noise. However, MC3T3-E1 osteoblasts showed noise-enhanced PGE_2 response, while MLO-Y4 osteocytes did not, compared to their responses to noise alone. The difference in responses by MLO-Y4 and MC3T3-E1 cells implied differences in stress-thresholds for the production of NO and PGE_2. Since NO and PGE_2 regulate bone formation as well as resorption, our results explain how noise might enhance the activity of osteocytes and osteoblasts *in vivo* in driving the mechanical adaptation of bone.

INTRODUCTION

Stochastic resonance (SR) is the phenomenon, in which non-linear systems show enhanced response at the output to noise-supplemented input signals. SR has been used to explain various phenomena in biological systems (1), including the activity of cells under microgravity (2). In another study, it was suggested that SR enhances bone formation (3). However, much is unknown about the role of noise for the response of different bone cell types to stress.

Living bone tissue is permeated by a fluid-filled microscopic network of lacunae and canaliculi. Bone cells are housed in lacunae, and are interconnected via gap-junctions connecting cellular extensions through the canaliculi (4-6). Bone is minutely deformed by mechanical loading due to exercise or normal daily activities (7). Although normal daily activities mostly induce strain deformations at 10 με (7), it is believed that strains to the order of 3000 με, mediate the flow of interstitial fluid in bone (8-10). Han et al. (11) suggested a strain amplification mechanism at the cellular level to explain the paradox of sustained bone health and the persistence of very small strains in normal daily activities (7). It is unknown whether bone cells use other amplification mechanisms for sensing small strains in bone. Nevertheless, fluid shear stress induced by the loading-mediated flow of interstitial fluid through the lacuno-canalicular network is a likely stimulus for bone cell adaptive responses (12-14).

Osteocytes, being most responsive to fluid shear stress (15, 16), are hypothesized to be the functional orchestrators of bone remodeling (5, 12). In response to fluid shear stress, osteocytes produce signaling molecules that are potent regulators of the activity of other bone cells, osteoblasts and osteoclasts (12, 15). The crucial balance between osteoblasts in depositing bone (osteoid and mineral), and that of osteoclasts in resorbing bone, determines the adaptive architecture of bone for efficient loading support (17-19). This phenomenon of bone cell activity has been hypothesized in the so-called “Bone mineralizing

unit" (BMU), where the release of signaling molecules in relation to local fluid shear stresses determine the mineralization process (20).

We have shown earlier that MC3T3-E1 osteoblasts' response to fluid shear stress is rate-dependent (21), and that this response necessitated an initial stress-kick (22). This suggests that bone cells have an initial activation barrier in terms of a stress-threshold. The necessity of an initial stress-kick for bone cells to respond to dynamic loading implies an essential non-linearity to the way bone cells respond to mechanical stress. This provides a cellular basis for stochastic resonance to occur in bone as a non-linear biological system.

Threshold detectors are unable to verify the presence of a signal below its threshold (1). The addition of noise at the input enables the detector to sense the small signal (1, 23, 24). A model for SR might use an information measure for the detectability of the input signal, at the output, by the addition of noise at the input. Models using the Fisher information as a measure for the detectability of the input signal in the presence of noise have been explored in detailed statistical analysis for single and multi-threshold systems for signals that are below or above the system threshold (25, 26).

This study investigated whether the activation of bone cells by a small periodic loading stimulus is enhanced by noise. A previous investigation on the NO response of MC3T3-E1 cells indicated a fluid shear stress rate of 0.11 Pa/s as a threshold for stimulation with an initial stress-kick due to the intial rise of the applied fluid shear stress from 0 to 0.7 Pa (21, 22). Here, we induced fluid shear stress regimes with an initial stress-kick due to a fluid shear stress rise from 0 to 1.4 Pa. To investigate the basic response of different bone cells to fluid shear stress, MLO-Y4 osteocytes and MC3T3-E1 osteoblasts were subjected to steady and dynamic fluid shear stress without noise. To investigate whether there is a difference to the response of different bone cell types to noisy stress, MLO-Y4 osteocytes and MC3T3-E1 osteoblasts, as models for primary osteocytes and osteoblasts, were subjected to a small periodic stimulus superposed with Gaussian white noise of different intensities. We then used

the results to propose differences in stress-thresholds for MLO-Y4 and MC3T3-E1 cells as modeled by noise-enhanced Fisher information about the small periodic stimulus input.

MATERIALS AND METHODS

Bone cell cultures

MLO-Y4 osteocytes were cultured up to near-confluency in 75 cm^2 cell culture flasks (Nunc, Roskilde, Denmark), using α-Modified Eagle's Medium (α-MEM; Gibco, Paisley, UK) supplemented with 5% fetal bovine serum (FBS; Gibco), 5% calf serum (CS; Gibco), penicillin (10 μg/ml) and streptomycin (10 μg/ml). The MLO-Y4 osteocytes were kindly provided by Dr. L. Bonewald (University of Missouri-Kansas City, Kansas City, MO, USA). MC3T3-E1 cells were cultured up to near-confluency in 75 cm^2 cell culture flasks (Nunc, Roskilde, Denmark), using α-Modified Eagle's Medium (α-MEM; Gibco, Paisley, UK) supplemented with 10% fetal bovine serum (FBS; Gibco), ascorbate (50 μg/ml; Merck, Darmstadt, Germany), β-glycerophosphate disodium salt hydrate (10 mM; Sigma, St. Louis, MO, USA), l-glutamine (300 μg/ml; Merck), gentamycine (50 μg/ml; Gibco), and fungizone (1.25 μg/ml; Gibco). The MC3T3-E1 osteoblasts were kindly provided by Dr. M. Kumegawa (Meikai University, Sakado, Japan). MLO-Y4 and MC3T3-E1 cells were then harvested and seeded at $1.5x10^5$ cells per polylysine-coated (50 μg/ml; poly-L-lysine hydrobromide; Sigma) glass slide (5 cm^2), and incubated overnight to promote cell attachment, at 37°C with 5% CO_2 in air, prior to fluid shear stress experimental treatment as described below.

Parallel-plate flow chamber *in vitro* sytem

To study the effect of noise on the response of bone cells to fluid shear stress, Gaussian white noise $\eta(t)$ was added to the applied fluid shear stress:

$$\tau(t) \approx \tau_c + \tau_d TK \sin(\omega t) + \eta(t) \quad [1]$$

where τ_c is a constant offset to the periodic forcing term with amplitude τ_d, superposed with $\eta(t)$, a zero-mean Gaussian white noise of intensity D (i.e., $<\eta(t)\eta(s)> = 2D\delta(t-s)$). The dimensionless product of the shear and flow factors TK attenuates the flow to 79% at frequencies above 89 Hz (for medium viscosity μ = 0.0069 Poise, chamber height h = 100 μm). The factor TK theoretically ensures flow frequencies up to 22.3 Hz without phase difference between the applied pressure gradient and flow (see (27) for a description of the parallel-plate flow chamber). Considering flow frequency limitations, the applied noise had a spectrum band reaching up to 22.3 Hz. The applied noise intensity D was chosen between 0 Pa to 1.4 Pa (Table 1).

Fluid shear stress application

Pulsating fluid shear stress was generated using a flow apparatus containing a parallel-plate flow chamber (PPFC) as described earlier (15, 21). Fluid shear stress was induced for 5 min on the monolayer of cells by circulating 5 ml of CO_2-independent medium (Gibco) containing 2% FBS for MC3T3-E1 cells, 1% FBS and 1% CS for MLO-Y4 cells, plus supplements as described above, using a computer-controlled micro-annular gear pump (developed by HNP Mikrosysteme GmbH, Parchim, Germany). Precise flow regimes (Table 1) were implemented at room temperature (22.5^{o}C) by controlling the pressure gradient using computer-mediated instrumentation (LabView^{TM}, National Instruments Corp., Austin, TX, USA). The flow was monitored online using a small animal blood flowmeter (T206, Transonic Systems Inc., Ithaca, NY, USA).

Table 1. *Data of applied pulsating fluid shear stress (PFSS) regimes*

D (Pa)	Mean Shear stress (Pa)	f (Hz)	A (Pa)	n (MLO-Y4)	n (MC3T3-E1)
0	1.4	0	0	9	6
0	1.4	9	0.12	10	6
0	1.4	9	1.4	6	6
0.25	1.4	0	0	6	--
0.70	1.4	0	0	--	6
0.04	1.4	9	0.12	4	5
0.07	1.4	9	0.12	5	5
0.11	1.4	9	0.12	4	6
0.14	1.4	9	0.12	7	7
0.25	1.4	9	0.12	5	5
0.32	1.4	9	0.12	5	5
0.42	1.4	9	0.12	5	5
0.70	1.4	9	0.12	4	5
1.09	1.4	9	0.12	4	5
1.40	1.4	9	0.12	5	6

f = frequency; A = amplitude; D = Noise intensity; n = number of experiments

Nitric oxide and prostaglandin E_2 determination

The conditioned medium was assayed for NO and prostaglandin E_2. NO was measured as nitrite (NO_2^-) accumulation in the conditioned medium, using Griess reagent (1% sulfanilamide, 0.1% naphtylethelene-diamine-dihydrochloride, and 2.5 M H_3PO_4). The absorbance was measured at 540 nm. NO concentrations were determined using a standard curve derived from known concentrations of $NaNO_2$ in non-conditioned culture medium. PGE_2 was measured in conditioned medium by an enzyme immunoassay (EIA) system (Amersham, Buckinghamshire, UK) using an antibody raised against mouse PGE_2. The absorbance was measured at 450 nm.

Stochastic resonance by noise-enhanced Fisher information

To capture the essential features of the observed accumulated release of NO and PGE_2 as associated to the information content at the output, we use the

Fisher information *I*, which is a parameter for the estimation of the input signal τ (see (25) for a statistical description):

$$I(\theta,\tau,\sigma)=\frac{\left[f\left(\frac{\theta-\tau}{\sigma}\right)\right]^2}{\sigma^2 F\left(\frac{\theta-\tau}{\sigma}\right)\left[1-F\left(\frac{\theta-\tau}{\sigma}\right)\right]} \qquad [2]$$

where θ is the threshold, τ the apparent input signal recognized by the bone cell. The noise is taken to have a Gaussian distribution with the standard deviation σ, which is $D^{1/2}$ with normalized density *f*, such that *F(x)*:

$$F(x)=\int_{-\infty}^{x} f(x)dx \qquad [3]$$

$$f(x)=(1/\sqrt{2\pi})\exp(-x^2/2) \qquad [4]$$

Our model takes the Fisher information as a measure for the ability of bone cells to detect the presence of a small periodic signal buried under noise. The released signaling molecules indicate the detectability of the input signal. The Fisher information predicts the presence of a peak response indicating the detectability of a signal with the presence of noise. Take for example, a signal = 1.0 (dimensionless), which is above the threshold; the peak in the Fisher information, increases as the threshold increases towards the signal value = 1.0 (Fig. 1). However, the peak decreases as the threshold increases further away, above the input signal value = 1.0. Note that for an input signal above the threshold, a higher peak response occurs at lower noise intensities for higher threshold values (Fig. 1).

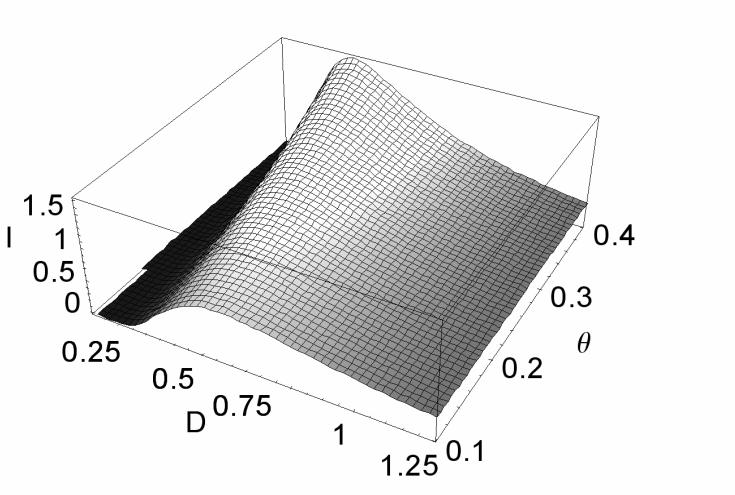

Figure 1. Fisher information *I*. The threshold θ is here taken to be above the input signal τ, that is, $\theta - \tau > 0$. D, noise intensity.

Statistics

Data were pooled from the results of at least four experiments for each fluid shear stress regime tested (Table 1). The effects of treatment with fluid shear stress were analyzed with the non-parametric Wilcoxon signed-rank sum test of the S-Plus 2000 package (release 1). Differences were considered significant at a p-value < 0.05.

RESULTS

Application of fluid flow for 5 min to the MLO-Y4 and MC3T3-E1 cells did not result in visible changes in cell shape or alignment of the cells in the direction of the fluid flow. No cells were removed by any of the fluid shear stress regimes, as assessed by visually inspecting the cultures before and after fluid shear stress treatment.

The NO released by MLO-Y4 cells and MC3T3-E1 in response to steady fluid shear stress compared to the dynamic fluid shear stress regimes were similar (Fig. 2a). However, MLO-Y4 cells released more NO in response to the 9 Hz hi regime (Table 1, Fig. 2a). MLO-Y4 and MC3T3-E1 cells released the highest amount of PGE_2 in response to the 9 Hz hi regime (Fig. 2b). PGE_2 release by MLO-Y4 cells was significantly higher compared to the response of MC3T3-E1 cells in response to steady fluid shear stress, as well as to dynamic fluid shear stress at both the 9 Hz lo and hi regimes (Fig. 2b).

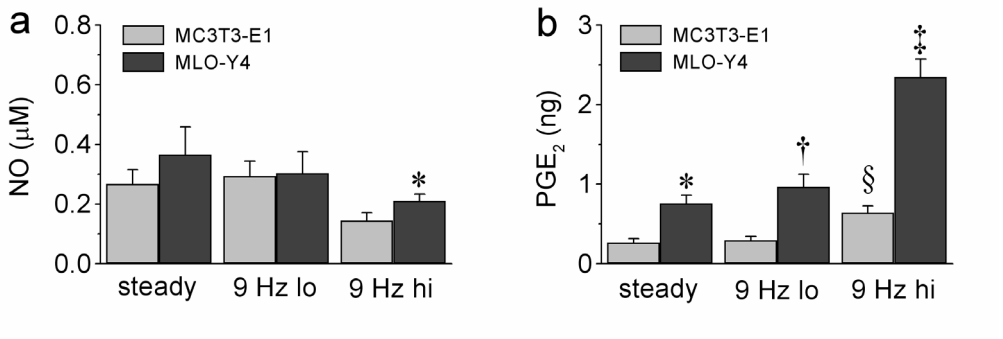

Figure 2. NO and PGE_2 production by MLO-Y4 and MC3T3-E1 cells. a. NO release by MLO-Y4 and MC3T3-E1 cells were similar in response to steady fluid shear stress (1.4 Pa) or 9 Hz lo regime (average 1.4 Pa, amplitude 0.12 Pa, frequency 9 Hz). NO release by MLO-Y4 cells was greater than the release by MC3T3-E1 cells at the 9 Hz hi regime (average 1.4 Pa, amplitude 1.4 Pa, frequency 9 Hz; $^*p < 0.029$). b. PGE_2 release by MLO-Y4 was higher than MC3T3-E1 cells in response to steady fluid shear stress (1.4 Pa), ($^*p < 0.016$), as well as, in response to 9 Hz lo regime (average 1.4 Pa, amplitude 0.12 Pa, frequency 9 Hz; at $^\dagger p < 0.001$) and in response to 9 Hz hi regime (average 1.4 Pa, amplitude 1.4 Pa, frequency 9 Hz; at $^\ddagger p < 0.016$). MLO-Y4 cell release of PGE_2 was most significant at the 9 Hz hi regime ($^\ddagger p < 0.016$; higher than the response at steady or 9 Hz lo regime). MC3T3-E1 cells release of PGE_2 was significant at the 9 Hz hi regime ($^\S p < 0.016$ greater than the response at 9 Hz lo regime; $p < 0.008$ greater than the response at steady fluid shear stress). Results are mean ± SEM.

The NO released by the MLO-Y4 cells showed an optimum response to noisy stress with an intensity of 0.25 Pa (Fig. 3a). The NO response to 0.25 Pa

was significantly higher than the response to a very low noise intensity, 0.04 Pa, and to high noise intensities from 0.42 Pa, to a very high noise intensity, 1.4 Pa (Fig. 3a). NO release by MC3T3-E1 cells did not significantly reach an optimum at any noise intensity, although the trend suggests an optimum at a noise intensity of 0.7 Pa (Fig. 3a). Only MLO-Y4 cells released NO with a definitive peak response to noise, but not MC3T3-E1 cells.

The NO released in response to 9 Hz lo regime at the optimum noise intensity (0.25 Pa) by MLO-Y4 cells was significantly higher (Fig. 3b) than the response to noise alone (0.25 Pa), as well as to 9 Hz lo superposed with a very high intensity (Fig 3b). However, this response was not higher than the NO release to the 9 Hz lo regime without noise. Similarly, NO release by MC3T3-E1 cells in response to 9 Hz lo regime at its optimum noise intensity (based on the trend, 0.70 Pa) was significantly different from the response to noise alone (noise intensity at 0.70 Pa; Fig. 3b). However, NO release by MC3T3-E1 osteoblasts, at the optimum noise level was not significantly higher than the release due to 9 Hz lo regime without noise, or with a very high noise (1.4 Pa) (Fig. 3b). Both MLO-Y4 and MC3T3-E1 cells released NO at their optimum noise level significantly higher than the response to noise alone.

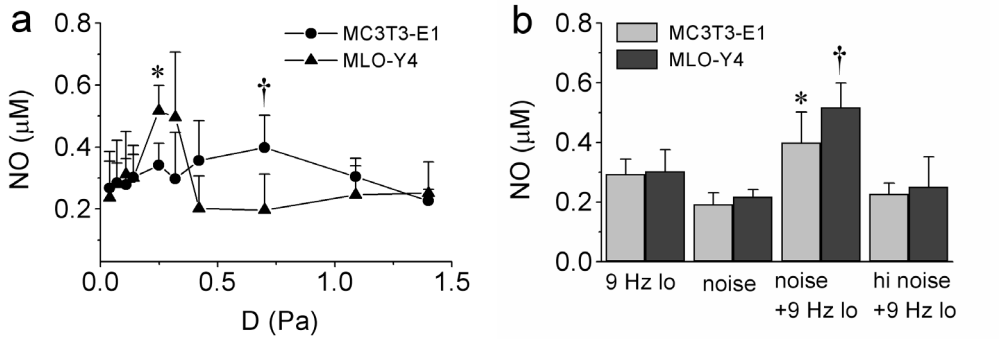

Figure 3. NO production by MLO-Y4 and MC3T3-E1 cells in response to noisy stress. a. NO release by MLO-Y4 cells in response to noisy stress was optimum at a noise intensity of 0.25 Pa (*$p < 0.047$, greater than the response at noise intensity 0.04 Pa; $p < 0.038$, greater than the response at noise intensity 1.40 Pa; $p< 0.023$, greater than the response at noise intensity 0.42 Pa). NO release by MC3T3-E1 cells in response to noisy stress does not result in a statistically significant optimum (†, probable peak at 0.70 Pa). b. NO release by MLO-Y4 cells at its optimum noise intensity (0.25 Pa noise intensity superposed with 9 Hz lo regime) is significantly larger than the response to noise alone (*$p < 0.030$, for 0.25 Pa noise alone) and to the highest noise intensity (*$p < 0.038$, for 1.4 Pa noise intensity superposed to 9 Hz lo). NO release by MC3T3-E1 cells at its possible optimum noise intensity (0.70 Pa noise intensity superposed with 9 Hz lo regime) is significantly higher than the response to noise alone (†$p < 0.030$, for 0.70 Pa noise alone), but not to the highest noise intensity (1.4 Pa noise intensity superposed to 9 Hz lo). Results are mean ± SEM.

The PGE_2 released by MLO-Y4 cells in response to noisy stress did not show a well defined peak response (Fig. 4a). However, the PGE_2 response of the MLO-Y4 cells to a noise intensity of 0.70 Pa was significantly higher than the response to its neighboring noise intensities 0.42 Pa and to 1.09 Pa (Fig. 4a). MC3T3-E1 cells released PGE_2 with a high level of variance in response to noise intensity of 0.42 Pa (Fig. 4a). This response is, however, not a statistically significant peak (Fig. 4a). Both cell types showed possible PGE_2 peak responses (Fig. 4a).

The PGE_2 released by MLO-Y4 cells at its possible optimum noise level, D = 0.70 Pa, was not significantly higher than the response to noise alone (D =

0.70 Pa), as well as to 9 Hz lo regime, or very high noise (Fig. 4b). However, MC3T3-E1 cells' release of PGE_2 at its supposed optimum (D = 0.42 Pa) was significantly higher than the response to noise alone (D= 0.42 Pa), and to 9 Hz lo regime without noise (Fig. 4b). Only MC3T3-E1 cells, but not MLO-Y4 cells, showed a peak PGE_2 response at the optimum noise that was significant compared to the response to noise alone.

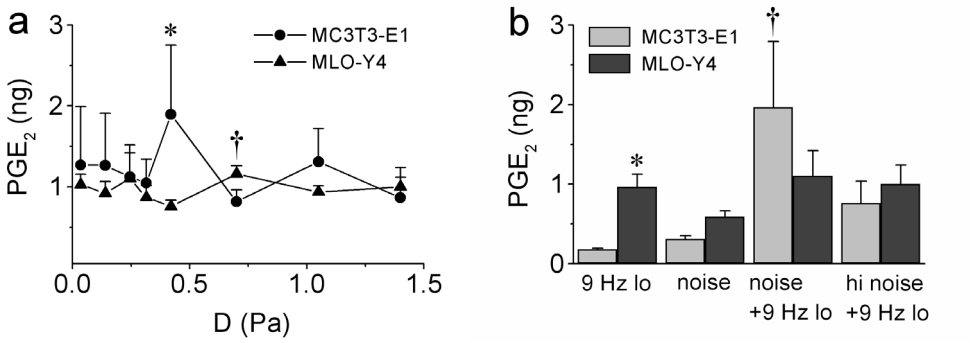

Figure 4. PGE_2 production by MLO-Y4 and MC3T3-E1 cells in response to noisy stress. a. MC3T3-E1 cells did not show a pronounced peak response to fluid shear stress although the trend implies a possible optimum at D =0.42 Pa (*$p < 0.06$ higher than response at D = 1.4 Pa). MLO-Y4 cells release PGE_2 with a locally pronounced peak at noise intensity D = 0.70 Pa, which is significantly higher than response at D = 0.42 Pa, $p < 0.029$, and than the response at D = 1.09 Pa, †$p< 0.029$. b. PGE_2 release by MLO-Y4 was higher than MC3T3-E1 cells in response to 9 Hz lo regime (average 1.4 Pa, amplitude 0.12 Pa, frequency 9 Hz; at *****$p < 0.001$). MC3T3-E1 cell release of PGE_2 at its supposed optimum (D = 0.42 Pa) was significantly larger than the response to noise alone (D= 0.42 Pa), and to 9 Hz lo regime without noise (†$p < 0.032$). PGE_2 release by MLO-Y4 cells at its possible optimum noise level, D = 0.70 Pa, was not significantly higher than the response to noise alone (D = 0.70 Pa). Results are mean ± SEM.

DISCUSSION

In this study, we used MLO-Y4 and MC3T3-E1 cells to model primary osteocytes and osteoblasts and found novel ways by which bone cells might

cooperate for influencing local changes in bone mass and structure. Our results confirmed that there is a fundamental difference in the response of osteocytes and osteoblasts in response to fluid shear stress (15), here modeled by MLO-Y4 osteocytes and MC3T3-E1 osteoblasts. While NO release by MLO-Y4 and MC3T3-E1 cells are similar in response to steady and a small dynamic fluid shear stress, PGE_2 release by MLO-Y4 cells was higher compared to that of MC3T3-E1 cells.

Previously, we showed a rate-dependent response to fluid shear stress, provided that the cells are "kicked" in a pre-conditioned state (22). In that study, bone cells were subjected to a sudden increase of fluid shear stress from 0 to 0.7 Pa (initial stress-kick). Our results here did not show a rate-dependent response to fluid shear stress in terms of NO production, because the initial stress-kick was high (increase from 0 to 1.4 Pa), and therefore induced a high NO baseline production. The high NO baseline production likely hid the rate-dependent response to fluid shear stress. We have shown in another study that the PGE_2 response to the fluid shear stress by MC3T3-E1 cells was not rate-dependent (28). In that study, bone cells were treated with regimes that have an initial stress-kick due to an initial fluid shear stress rise from 0 to 0.6 Pa. Here, we showed that the response to PGE_2 production for both cell types, was apparently rate dependent. Furthermore, MLO-Y4 cells released more PGE_2 compared to MC3T3-E1 cells. This difference can be attributed to our use of a higher intial stress-kick, which is due to an intial fluid shear stress rise from 0 to 1.4 Pa. It would seem that a fluid shear stress rate-dependent production of PGE_2 for MC3T3-E1 or MLO-Y4 cells requires a higher initial stress-kick (0 to 1.4 Pa) than what NO production requires (0 to 0.7 Pa). Thus, the initial threshold barrier for NO production is lower than the initial threshold barrier for PGE_2 production for both cell types (Table 2).

Table 2. *Stress-threshold comparison between cells*

	NO	PGE_2
MLO-Y4	Higher threshold	Lower threshold
MC3T3-E1	Lower threshold	Higher threshold

The stress-threshold for NO is lower than PGE_2 for both MLO-Y4 and MC3T3-E1 cells

Our results showed different responsiveness to noisy stress, which suggests a difference in the stress-thresholds of the two bone cell types for NO production. The difference in stress-thresholds is predicted by our model using the Fisher information which fits the re-scaled peak NO responses of MLO-Y4 and MC3T3-E1 cells (Fig. 5). MC3T3-E1 cells did not show a definitive peak NO release because MC3T3-E1 cells have a lower stress-threshold for NO release compared to MLO-Y4 cells (Table 2). Although noise seems to enhance NO production by MLO-Y4 cells, our results indicated that noise alone, even at the optimum intensity (0.24 Pa), does not enhance NO production as noisy stress with a small periodic stimulus. This suggests that noisy stress conditions are stimulatory only in the presence of periodic loading as would be expected in exercise or sports with repetitive motions.

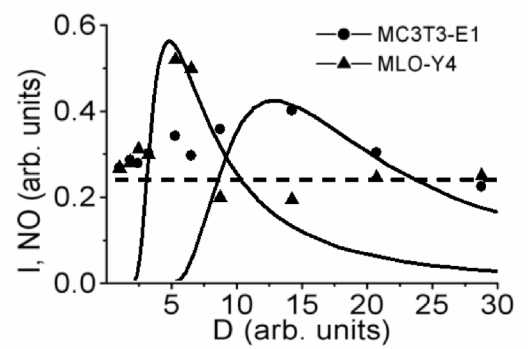

Figure 5. NO production by MLO-Y4 and MC3T3-E1 cells as a function of noise intensity plotted with the predicted peak from the Fisher Information, predicted with the input signal greater than the stress-threshold. Dashed line indicates NO production baseline. Arb. units., arbitrary units.

Previously, it has been shown that primary osteoctyes produces more PGE_2 in response to fluid shear stress compared to primary osteoblasts and periosteal fibroblasts (29), confirming MLO-Y4 and MC3T3-E1 cells as plausible models for primary bone cells (osteocytes and osteoblasts, respectively). PGE_2 production in response to noisy stress was more significant for MC3T3-E1 cells compared to MLO-Y4 cells. No indicative PGE_2 peak was observed for both cell types, possibly because the small periodic signal (9 Hz lo regime), although above the PGE_2 stress-threshold, is too far from the PGE_2 stress-threshold. Using the Fisher information to predict the presence of a peak response to noise, it is possible that MC3T3-E1 cells have a higher stress-threshold for PGE_2 production compared to MLO-Y4 cells (Table 2). It is possible that MLO-Y4 cells are more sensitive to stress in terms of PGE_2 production, but MC3T3-E1 cells are more sensitive to stress in terms of NO production (as discussed above, see Table 2). While it might be observed that osteocytes produce more signaling molecules compared to osteoblasts or periosteal fibroblasts in response to stress (15, 29, 30), the loading threshold for specific signaling molecules (*e.g.* PGE_2, in this study) is not necessarily lower. MLO-Y4 cells produced more signaling molecules in response to fluid shear stress, compared to MC3T3-E1 cells. However, MLO-Y4 cells have a lower PGE_2 stress-threshold compared to MC3T3-E1 cells, while MC3T3-E1 cells have a lower NO stress-threshold compared to MLO-Y4 cells (Table 2).

Both NO and PGE_2 are important intercellular messenger molecules for bone cells. Studies on whole animals have show that they play a key role in the mechanical adaptation of bone, because inhibiting their synthesis inhibits bone adaptation to loading (31-33). NO is known to drive away osteoclasts *in vitro* (34). On the other hand, PGE_2 increases mRNA levels of osteoprotegerin ligand (OPG-L)/osteoclast differentiation factor (ODF), from osteoblastic lineage cells. OPG-L/ODF stimulates osteoclast differentiation and activity, further inhibiting osteoclast apoptosis (35). NO and PGE_2 regulate the activity of osteoclasts (34, 36). It would seem that osteocytes, deep in the bone matrix, drive away osteoclasts, preventing bone resorption in their regions by

producing NO at high stress conditions (since MLO-Y4 cells as model for osteocytes have a higher stress-threshold for NO production compared to MC3T3-E1 cells as model for osteoblasts; Table 2). At low stress conditions, our results indicate that osteocytes might promote the activity of osteoclasts by producing more PGE_2 and lesser NO (since the stress-threshold for PGE_2 is lower compared to NO for MLO-Y4 cells; Table 2). Since MC3T3-E1 cells produce less NO and PGE_2 compared to MLO-Y4 cells, given the same loading conditions, it would seem that *in vivo*, osteocytes are indeed more responsible for directing the activity of osteoclasts. However, osteoblasts might produce high levels of NO locally under conditions of high stress to drive away osteoclasts, towards regions of low stress where osteocytes are still able to produce PGE_2 to promote osteoclast activity.

The possibility of SR in bone cells further suggests that osteocytes and osteoblasts take advantage of noisy stresses as an alternative mechanism for the adaptation of bone to mechanical loading by tuning their different peak responses for specific signaling molecules. This explains the osteogenic benefits of dynamic stress to functional bone adaptation to mechanical loading. By a tuned nitric oxide and prostaglandin E_2 response to noisy stress, it is possible that osteocytes and osteoblasts locally recruit or inhibit osteoclasts, for a functional manipulation of bone mass and architecture.

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