OSTEOBLASTS TUNE THEIR MECHANICAL BEHAVIOR TO THE MAGNITUDE OF HABITUAL MECHANICAL LOADING

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Abstract: Numerous cellular biochemical responses to mechanical loading are transient, indicating a cell's ability to adapt its behavior to a new mechanical environment. Since load-induced cellular deformation can initiate these biochemical responses, we investigated the adaptation of whole-cell mechanical behavior, i.e. cellular deformability, in response to mechanical loading for osteoblastic cells. Confluent cell cultures were subjected to 1 Pa or 2 Pa flow-induced shear stress for two hours. Whole-cell mechanical behavior was then measured for individual cells using an atomic force microscope. Compared to cells maintained in static conditions, whole-cell mechanical behavior, as characterized by a parallel-spring recruitment model, was 1.36-fold ($p = 0.001$) and 1.70-fold ($p < 0.001$) stiffer for cells exposed to 1-Pa and 2-Pa shear loading, respectively. The increase in cell stiffness was not a short-term effect as mechanical behavior was not altered in either flow-loaded group for 70 minutes after flow was terminated ($p \geq 0.25$). These findings support the concept that cells undergo a purposed response so as to become mechanically tuned to their mechanical environment. Accordingly, cellular mechanical adaptation may play a significant role in regulation of cellular mechanosensitivity and the related effects on tissue structure and function.

Introduction

Numerous cell phenotypes, including osteocytes, osteoblasts, endothelial cells, chondrocytes, fibroblasts, and smooth muscle cells, respond to mechanical loading with various biochemical responses such as an increase in intracellular calcium [1], production of nitric oxide [2] and prostaglandins [3], and expression of various transcription factors [4,5] and extracellular matrix proteins [6]. However, many of these biochemical responses are transient as levels can decline — often back to baseline values — one to several hours after initiation of loading [2-4,6]. This finding suggests that mechanosensory cells adapt their activity based on their predominant mechanical environment [7], i.e. cells can become "tuned" to their mechanical environment. Cellular activity determines tissue structure, and, indeed, tissue adaptation to mechanical loading has been seen in bone, tendon, ligament, muscle, skin, and lung tissue [8]. For bone tissue in particular, similar to transient cellular responses, addition of bone tissue in response to increased mechanical loading diminishes with extended exposure to loading [9]. The mechanisms responsible for controlling cellular and tissue adaptation are poorly understood. Accordingly, improved understanding of bone cell mechanical behavior and its adaptation to mechanical loading could provide unique insight into bone remodeling mechanisms, diseases in which they are affected, as well as bone tissue engineering.

While the effects of mechanical loading on biochemical signaling have been extensively studied for numerous cell types, the effects of mechanical loading on subsequent cellular mechanical behavior, e.g. cellular deformability, have received limited attention. Studies have shown that fibroblasts [10], endothelial cells [11,12], and smooth muscle cells [13] are capable of mechanical stiffening in response to increased mechanical loading. Changes in mechanical behavior have been first observed anywhere from 30 minutes [10,12] to 24 hours [11] after initiation of loading. The stiffness increase was qualitatively determined to last for at least six hours after 24 hours of shear loading [12]. An adaptive response, i.e. cell stiffness dependent on loading magnitude, has only been reported for endothelial cells after long-term exposure (>12 hours) to physiological levels of mechanical loading [12], whereas short-term mechanical adaptation appears to play a key role in bone cell activity and, consequently, in bone adaptation.

It has been hypothesized that the transient nature of many cellular biochemical responses to mechanical loading is a result of adaptation in cellular mechanical behavior, whereby the cell becomes mechanically tuned to its mechanical environment [14,15]. This may be particularly important for bone cells as they are constantly remodeling bone tissue. However, cellular mechanical adaptation has only been investigated for endothelial cells. It has not been determined if such an adaptive response occurs for additional cell types that also are routinely subjected to mechanical loading *in vivo*. In addition, the duration of cellular stiffening after

ISSN: 1727-1983

increased loading is terminated has not been investigated in detail. Because *in vivo* loading can invoke a mechanical response that depends on the structure of the entire cell [16], the overall goal of this study was to investigate the adaptation in whole-cell mechanical behavior for osteoblastic cells in response to mechanical loading. The specific objectives were to investigate: 1) the effects of loading magnitude on whole-cell mechanical behavior and 2) the short-term, temporal changes in whole-cell mechanical behavior after loading is terminated.

Materials and Methods

MC3T3-E1 osteoblastic cells were cultured under standard culture conditions (37°C, 5% CO_2) in α-MEM (Cellgro, Herndon, VA) supplemented with 10% fetal bovine serum (FBS, GIBCO, Gaithersburg, MD) and 1% penicillin/streptomycin (P/S, Cellgro). Cells were plated on 25-mm diameter glass coverslips (Fisher Scientific, Pittsburg, PA) coated with collagen I (BD Biosciences, Bedford, MA) to achieve a tight-packed confluence on the day of experimentation. For flow exposure, *flow media* consisted of CO_2 independent medium (GIBCO) supplemented with 1% FBS, 1% P/S, and 200μM L-glutamine (GIBCO). Due to evaporation during atomic force microscope (AFM) experimentation, the flow media was diluted for AFM loading with distilled water (*AFM media*) to maintain osmolality in the acceptable range of 260–320 mOsm/kg [17] throughout experimentation.

Cells were exposed to fluid flow-induced shear stress using a parallel plate flow chamber (Fig. 1). The flow chamber was modified from a standard design [18] by boring a shallow impression in the lower piece, which allowed for addition of the cell coverslip without disrupting the laminar flow profile. A peristaltic pump (Cole-Palmer Instrument Company, Vernon Hills, IL) was used to replenish the media in an upper reservoir, which provided steady, gravity-driven flow through the chamber. The pump flow rate was set to achieve desired shear stress according to the equation

$$Q = \frac{bh^2\tau}{6\mu}, \qquad (1)$$

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

An AFM with a temperature-controlled (37 ± 0.04°C) stage (BioScope, Veeco Metrology, Santa Barbara, CA) was mounted on an inverted microscope (TE300, Nikon, Melville, NY). The entire system was positioned on a vibration isolation table (9101, Kinetic Systems, Boston, MA), and the AFM head and stage were enclosed within an acrylic box for isolation from acoustic noise and air currents. V-shaped, gold-coated silicon nitride cantilevers, having a length of 200-μm (Veeco Metrology), were used in all experiments. A polystyrene microsphere (diameter = 25.2 ± 1.0 μm, Duke Scientific, Palo Alto, CA) was glued onto the underside of each cantilever. For each cantilever, the spring constant was determined three times using the thermal fluctuation method [19], and the average value was used. In total, ten cantilevers, having an average spring constant of 81.9 ± 6.0 mN/m (mean ± S.D.), were used for experiments on 17 cell cultures (2–12 cells tested per culture dish).

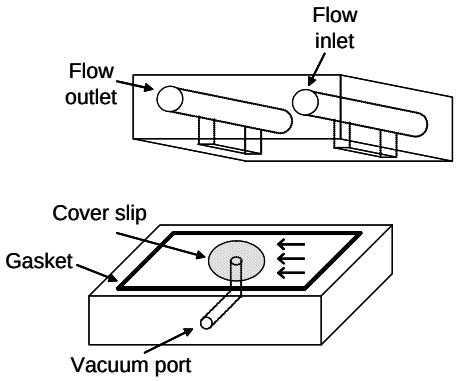

Figure 1: Flow chamber for parallel plate flow system. Cells were seeded on the glass coverslip, which was held in place by vacuum pressure. The upper and lower pieces were clamped together, and the flow field was sealed by the gasket. Arrows indicate flow direction.

Cell coverslips were separated into the following loading groups: Static culture conditions, 1 Pa shear stress, and 2 Pa shear stress. All coverslips were placed in the flow chamber. For the S*tatic* group, the coverslip was immediately removed from the flow chamber. For the *1 Pa* and *2 Pa* groups, cells were exposed to steady fluid flow at 1 and 2 Pa shear stress, respectively, for two hours. Once removed from the flow chamber, the coverslip was placed in a 60-mm culture dish with AFM media at 37°C.

Mechanical testing of individual cells was conducted as described previously [20]. Briefly, immediately after exposure to fluid flow, mechanical testing was performed directly above the cell nucleus using a triangular wave function with an amplitude of 2.0 μm at a loading rate of 4.0 μm/s. First, five calibration loading cycles were conducted. The first two cycles were preformed for pre-conditioning and were not analyzed. The loading portion of each of the last three cycles was immediately analyzed (MATLAB 6.1, The Mathworks Inc, Natick, MA) to determine the cantilever deflection required to obtain a cellular deformation of 600 nm. The cell was then cyclically loaded for 12 cycles to a deformation of 600 nm. The complete procedure starting with the five calibration loading cycles through the 12 trigger mode loading cycles is referred to as a single *mechanical testing session*. Multiple cells per coverslip were mechanically tested. After removal from the flow chamber, cells were mechanically tested for up to 90 minutes. The duration between removal from the flow chamber and measurement of mechanical behavior was recorded for each cell.

ISSN: 1727-1983

The cantilever was intended to lose contact with the cell during the unloading portion of each loading cycle. The point of initial probe/cell contact was determined for each loading cycle as previously described [20]. The point of contact was used with the cantilever base position and cantilever deflection to determine cellular deformation and applied force. The cellular force-deformation curve was then constructed for the loading portion of each loading cycle. To account for variations in maximum cellular deformation between loading cycles, each force-deformation curve was truncated to the cellular deformation range of 0–500 nm. Of the final 12 loading cycles in each mechanical testing session, the first two cycles were performed for pre-conditioning. The last ten cycles were evaluated to determine cellular mechanical behavior.

A parallel-spring recruitment (PSR) model [20], which consists of an infinitely large number of parallel springs that have the same spring constant value but are sequentially recruited as deformation increases, was used to describe the force-deformation response. The Levenberg-Marquardt algorithm was used to fit each force-deformation curve to the model according to the equation

$$F = \kappa_{cell}\delta^2, \qquad (2)$$

where F is the applied force, δ is the cellular deformation, and κ_{cell} is a constant that is characteristic of the cellular mechanical behavior. Mean values of κ_{cell} for each cell were used in all statistical analyses.

All statistical analyses were performed in JMP 2.0 (SAS Institute, Cary, NC). Using Grubbs' test on the mean κ_{cell} for each cell, three outliers were detected and removed. Thus, in total, we obtained valid measurements from 135 of the 138 cells that were tested (n = 45 cells per loading group). Bartlett's test for homogeneity of variances showed the κ_{cell} results to be heteroscedastic (p = 0.003). Therefore, nonparametric statistical tests were used. First, Kendall's coefficient of rank correlation was used to determine if load-induced changes in whole-cell mechanical behavior were altered over time after flow exposure. This correlation test was performed on each loading group separately. For all groups, κ_{cell} was not correlated with time. Therefore, to determine the effects of the magnitude of mechanical loading on whole-cell mechanical behavior, we evaluated differences in κ_{cell} between loading groups. Differences in κ_{cell} were assessed using the Kruskal-Wallis test followed by the nonparametric equivalent of the Student-Newman-Keuls multiple comparison test [21]. In all analyses, statistical significance was determined using $\alpha = 0.05$.

Results

The PSR model accurately described cellular mechanical behavior, with an average coefficient of determination of at least 0.99 for all loading groups. Whole-cell mechanical behavior, as characterized by κ_{cell}, was significantly different between all loading groups (Fig. 2A). Compared to static cells, κ_{cell} was 1.36-fold (p = 0.001) and 1.70-fold (p <0.001) greater for the 1 Pa and 2 Pa flow groups, respectively. Accordingly, cellular reaction forces were substantially different between cell groups (Fig. 2B*)*. Considerable inter-cell variability in whole-cell mechanical behavior was also observed within all cell groups as the average coefficient of variation (COV = S.D./mean) for κ_{cell} was in the range 41–51%.

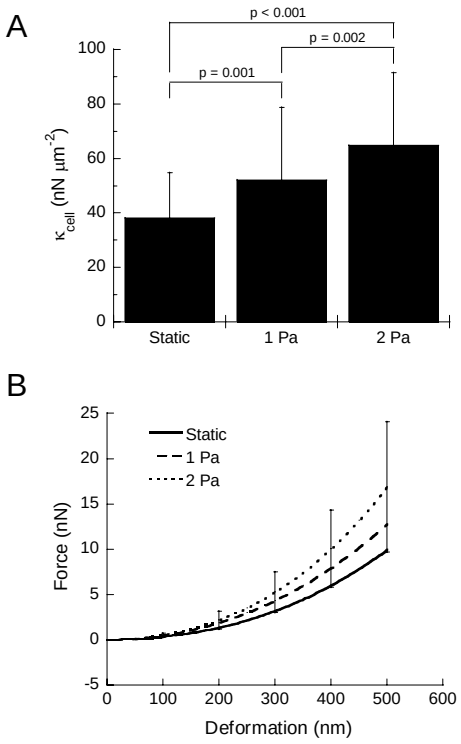

Figure 2: Measured whole-cell mechanical behavior. A) κ_{cell} values. Multiple comparison tests showed significant differences between all loading groups. Error bars represent S.D. B) Mean force-deformation responses. Error bars, representing S.D., are shown for the 2 Pa loading group.

The length of time between removal from the flow chamber and measurement of mechanical behavior with the AFM did not affect measured whole-cell mechanical behavior (Fig. 3). Correlations between κ_{cell} and time after removal were not significant for the Static (p = 0.75), 1 Pa (p = 0.58), and the 2 Pa (p = 0.25) groups.

Discussion

Numerous cellular biochemical responses to mechanical loading are transient, indicating a cell's ability to adapt its behavior to a habitual mechanical environment. Since load-induced cellular deformation can produce these biochemical responses [14,22,23], we sought to test the hypothesis that a cell becomes mechanically tuned to its predominant mechanical environment. Whole-cell mechanical behavior, as characterized by the PSR model, increased with an increase in the conditioning load magnitude. The

ISSN: 1727-1983

increased stiffness was not a short-term effect because, for 70 minutes after flow-induced loading was terminated, there was no statistical correlation between measured mechanical behavior and duration after termination of flow for either flow group. Taken together, these results indicate that the osteoblastic cell is capable of a purposed adaptation of its mechanical behavior in response to changes in its habitual mechanical loading. This finding provides valuable insight into the control of cellular mechanotransduction and the resulting effects on tissue structure.

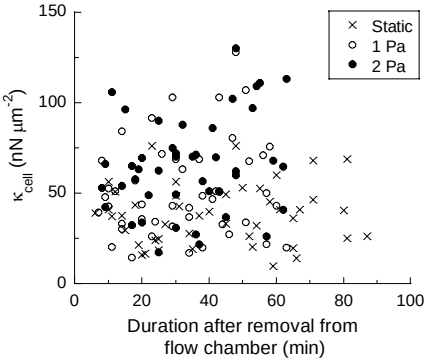

Figure 3: Effect of duration after flow exposure on whole-cell mechanical behavior. Separate analyses for each loading group showed no correlation between κ_{cell} and duration after removal from the flow chamber.

Several aspects of this study support the validity of the results. First, in accordance with published guidelines [20], to account for measurement uncertainties ten measurements were taken per cell, and the average value was used for analyses. Also, a sufficiently large sample size was used to provide high statistical power in all analyses. Second, with the goal of investigating whole-cell mechanical behavior, the microsphere probe was large enough to ensure measurement of this structural behavior [24]. And finally, the flow-induced shear stress magnitudes were in the range expected to be experienced *in vivo* during routine locomotion by osteocytes (0.8–3.0 Pa) [25], which are terminally differentiated osteoblasts. Similarly, the imposed cellular deformation under AFM loading was likely comparable to normal *in vivo* levels because the maximum deformation was in the range that can invoke an intracellular calcium response [1].

Despite these strengths, two caveats are noted. First, while the osteoblastic cells were loaded via steady fluid flow, it has been hypothesized that bone cells are primarily loaded *in vivo* via oscillatory flow [26]. Nonetheless, steady flow did induce changes in mechanical behavior. Based on previous reports of cellular stiffening [10,11,13], it appears that the cellular mechanisms of mechanical adaptation are independent of habitual loading mode and, thus, similar results can be expected for oscillatory flow. Second, characterization of the force-deformation response with the PSR model does not account for probe/cell contact area. Differences in mechanical behavior between cells could alter the contact area during loading, which could affect the resulting force-deformation responses. However, consistent shape of the force-deformation responses across loading groups is indicative of similar contact mechanics [24]. In addition, a large microsphere probe was used, which produces a large probe/cell contact area, so small deviations in contact area should not have a substantial effect on measured mechanical behavior.

The observed whole-cell mechanical behavior and variability in this behavior within each loading group are consistent with previous reports. Using the same loading protocol, confluent MC3T3-E1 osteoblastic cells showed a κ_{cell} value of 42.2 ± 15.1 nN μm^{-2} (mean ± S.D.) [20] compared to 38.2 ± 16.5 nN μm^{-2} in this study. The three loading groups showed COV values for κ_{cell} in the range of 41–51%. This is at the lower end of the variability in whole-cell mechanical behavior parameters observed in AFM studies (COV = 36–103%) [20,27] and observed in micropipette aspiration studies (COV = 30–128%) [28,29].

The mechanical adaptation observed for osteoblastic cells was similar to that reported for endothelial cells. After a 24-hr exposure to 2-Pa shear loading, whole-cell mechanical behavior of endothelial cells increased 2-fold as measured by micropipette aspiration [11]. In this study, a 2-hr exposure to 2-Pa shear stress loading produced a 1.7-fold increase in whole-cell mechanical behavior for osteoblastic cells. Moreover, after 24 hours of shear loading, endothelial cells exposed to a 3-Pa shear stress were 1.8-fold stiffer than cells exposed to a 1-Pa shear stress [12]. Our results show a linear increase in κ_{cell} with shear stress magnitude, which, when extrapolating the results, indicates a 1.5-fold increase in mechanical behavior with an increase in shear stress from 1 Pa to 3 Pa.

Our results indicate that cellular mechanical adaptation is not a short-term response. Cells were mechanically tested up to 70 minutes after concluding flow exposure, and there was no statistical correlation between κ_{cell} and lag time before measurement of mechanical behavior. In our laboratory, preliminary immunofluorescence imaging of the cytoskeleton indicates that cytoskeletal reorganization is also a lasting effect of mechanical loading. Study of tissue-level bone adaptation has shown that load-induced tissue addition is enhanced if loading sessions are separated by a recovery period of three hours or more [9]. Taken together, these findings point to the association between cellular mechanical adaptation and cellular mechanosensitivity adaptation, the effects of which appear to be reversed over the course of several hours after loading conditions return to previous levels.

Because the cytoskeleton plays a major rule in whole-cell mechanical behavior [14,16,27], the force-deformation responses provide insight into similarities and differences in cytoskeletal microstructure between cells from the three loading groups. The comparable shape of the force-deformation responses across loading groups indicates a consistent general cytoskeletal

ISSN: 1727-1983

structure that is the source of the nonlinear response [20,30]. However, absolute differences in the responses (Fig. 2B) also point to differences in the cytoskeletal microstructure between cell groups. One potential microstructural difference relates to filament crosslinking. Loaded cells undergo cytoskeletal reorganization whereby filament crosslinking increases [4,31]. Crosslinking can both increase the overall rigidity of the cell as a structure and enhance load transfer within the cell so that a greater portion of the cell is resisting deformation. Another potential microstructural modification is an increase in internal tension within the actomyosin network. The prestressed cable network model of the cytoskeleton predicts such an increase in cellular mechanical behavior with increased internal tension [30].

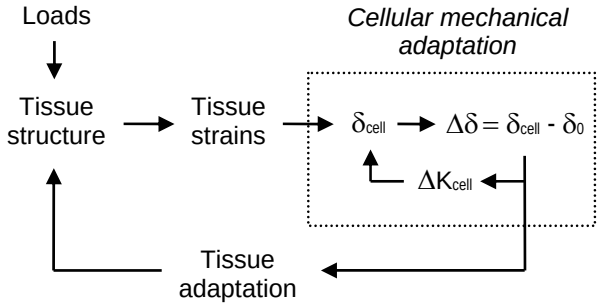

Figure 4: Illustration of cellular mechanostat feedback system for bone adaptation. The feedback loop for cellular mechanical adaptation is inserted into the tissue-level Mechanostat Theory. Whole-bone and tissue-level loading produce cellular deformation, δ. If a critical cellular deformation, δ_0, is reached (high-magnitude loading) or not attained (low-magnitude loading), both the cellular stiffness, K_{cell}, and tissue mechanical properties will be modified. These modifications then influence subsequent cellular deformation. Cellular mechanical adaptation can take place over minutes to hours, while tissue adaptation takes weeks to months.

The Mechanostat Theory [32] and the Principle of Cellular Accommodation [7] have been used to effectively describe bone adaptation at the tissue level. However, the Mechanostat Theory is solely a tissue-level model, while the Principle of Cellular Accommodation does not address the mechanisms of adaptation in cellular mechanosensitivity. Here, we propose modification of a more complete description of bone adaptation [33], whereby cellular mechanical adaptation is incorporated into the Mechanostat Theory (Fig. 4). The cellular mechanostat model contains two negative feedback loops, in which the control loop for cellular mechanical adaptation is embedded within the control loop for tissue adaptation. In this model, whole-bone loading produces tissue strains, which, in turn, result in cellular deformation. If a critical deformation is reached (high-magnitude loading) or failed to be reached (low-magnitude loading), internal biochemical responses can be initiated through various mechanisms [14,22]. Then, both cellular and tissue mechanical adaptation are initiated. Modifications of cellular and tissue mechanical properties alter the effects of subsequent mechanical loading on tissue and cellular deformations. Thus, cellular mechanical adaptation is the means by which the magnitude of mechanical stimulus that is required to initiate a biochemical response, i.e. setpoint stimulus for the feedback system, is altered. It is important to note that cellular mechanical adaptation can take place within minutes to several hours of changes in habitual mechanical loading, while tissue adaptation can take a month or more to occur [34]. Thus, it is likely that tissue adaptation is still underway after cellular mechanical adaptation has stopped the release of biochemical factors that would initiate further bone addition or removal.

Finally, although a magnitude-dependent increase in whole-cell stiffness was observed for shear loading, further investigation is required to confirm the comprehensive nature of cellular mechanical adaptation. After loading is terminated, a complete reversal of the stiffening response is required to indicate a truly adaptive response. In addition, given sufficient time to adapt, cellular mechanical behavior should not be history-dependent, i.e. the current mechanical behavior should only be dependent on the current mechanical environment. Further research into cellular mechanical adaptation, biochemical responses, and mechanosensitivity adaptation can validate the proposed relationships between these mechanisms and elucidate how they affect tissue adaptation. This knowledge has great potential for improving understanding tissue structure, function, development, adaptation, and the diseases that affect them, which have huge economic and social costs.

Acknowledgements

Funding for this study was provided by the National Science Foundation (BES-0201951) and the Whitaker Foundation Graduate Fellowship Program (WMJ).

References

[1] CHARRAS G. T., HORTON M. A. (2002): 'Determination of cellular strains by combined atomic force microscopy and finite element modeling', *Biophys. J.*, **83,** pp.858-79

[2] KLEIN-NULEND J., HELFRICH M. H., STERCK J. G., MACPHERSON H., JOLDERSMA M., RALSTON S. H., SEMEINS C. M., BURGER E. H. (1998): 'Nitric oxide response to shear stress by human bone cell cultures is endothelial nitric oxide synthase dependent', *Biochem. Biophys. Res. Commun.*, **250,** pp.108-14

[3] NAUMAN E. A., SATCHER R. L., KEAVENY T. M., HALLORAN B. P., BIKLE D. D. (2001): 'Osteoblasts respond to pulsatile fluid flow with short-term

ISSN: 1727-1983

increases in PGE(2) but no change in mineralization', *J. Appl. Physiol.*, **90,** pp.1849-54

[4] CHEN N. X., RYDER K. D., PAVALKO F. M., TURNER C. H., BURR D. B., QIU J., DUNCAN R. L. (2000): 'Ca(2+) regulates fluid shear-induced cytoskeletal reorganization and gene expression in osteoblasts', *Am. J. Physiol. Cell Physiol.*, **278,** pp.C989-97

[5] ADAM R. M., EATON S. H., ESTRADA C., NIMGAONKAR A., SHIH S. C., SMITH L. E., KOHANE I. S., BAGLI D., FREEMAN M. R. (2004): 'Mechanical stretch is a highly selective regulator of gene expression in human bladder smooth muscle cells', *Physiol. Genomics*, **20,** pp.36-44

[6] BREEN E. C. (2000): 'Mechanical strain increases type I collagen expression in pulmonary fibroblasts in vitro', *J. Appl. Physiol.*, **88,** pp.203-9

[7] TURNER C. H. (1999): 'Toward a mathematical description of bone biology: the principle of cellular accommodation', *Calcif. Tissue Int.*, **65,** pp.466-71

[8] FUNG Y. C. (1990): 'Biomechanics: Motion, Flow, Stress, and Growth', (Springer-Verlag, New York)

[9] ROBLING A. G., HINANT F. M., BURR D. B., TURNER C. H. (2002): 'Shorter, more frequent mechanical loading sessions enhance bone mass', *Med. Sci. Sports Exerc.*, **34,** pp.196-202

[10] GLOGAUER M., ARORA P., YAO G., SOKHOLOV I., FERRIER J., MCCULLOCH C. A. (1997): 'Calcium ions and tyrosine phosphorylation interact coordinately with actin to regulate cytoprotective responses to stretching', *J. Cell Sci.*, **110,** pp.11-21

[11] SATO M., OHSHIMA N., NEREM R. M. (1996): 'Viscoelastic properties of cultured porcine aortic endothelial cells exposed to shear stress', *J. Biomech.*, **29,** pp.461-67

[12] SATO M., LEVESQUE M. J., NEREM R. M. (1987): 'Micropipette aspiration of cultured bovine aortic endothelial cells exposed to shear stress', *Arteriosclerosis*, **7,** pp.276-86

[13] SMITH P. G., DENG L., FREDBERG J. J., MAKSYM G. N. (2003): 'Mechanical strain increases cell stiffness through cytoskeletal filament reorganization', *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **285,** pp.L456-63

[14] JANMEY P. A. (1998): 'The cytoskeleton and cell signaling: component localization and mechanical coupling', *Physiol. Rev.*, **78,** pp.763-81

[15] TURNER C. H. (1998): 'Three rules for bone adaptation to mechanical stimuli', *Bone*, **23,** pp.399-407

[16] MANIOTIS A. J., CHEN C. S., INGBER D. E. (1997): 'Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure', *Proc. Natl. Acad. Sci. USA*, **94,** pp.849-54

[17] FRESHNEY R. I. (2000): 'Culture of Animal Cells', (Wiley-Liss, New York)

[18] NAUMAN E. A., RISIC K. J., KEAVENY T. M., SATCHER R. L. (1999): 'Quantitative assessment of steady and pulsatile flow fields in a parallel plate flow chamber', *Ann. Biomed. Eng.*, **27,** pp.194-99

[19] BUTT H. J., JASCHKE M. (1995): 'Calculation of thermal noise in atomic force microscopy', *Nanotechnology*, **6,** pp.1-7

[20] JAASMA M. J., JACKSON W. M., KEAVENY T. M. (2005): 'Measurement and characterization of whole-cell mechanical behavior', *Ann. Biomed. Eng.*, **In press**

[21] ZAR J. H. (1984): 'Biostatistical Analysis', (Prentice-Hall, Englewood Cliffs, New Jersey)

[22] CHEN C. S., INGBER D. E. (1999): 'Tensegrity and mechanoregulation: from skeleton to cytoskeleton', *Osteoarthritis Cartilage*, **7,** pp.81-94

[23] KO K. S., MCCULLOCH C. A. (2000): 'Partners in protection: interdependence of cytoskeleton and plasma membrane in adaptations to applied forces', *J. Membr. Biol.*, **174,** pp.85-95

[24] JAASMA M. J., JACKSON W. M., KEAVENY T. M. (2005): 'Multi-scale cellular mechanical behavior', Spring Meeting of the Northern Ireland Biomedical Engineering Society, Belfast, UK, pp.11

[25] WEINBAUM S., COWIN S. C., ZENG Y. (1994): 'A model for the excitation of osteocytes by mechanical loading-induced bone fluid shear stresses', *J. Biomech.*, **27,** pp.339-60

[26] JACOBS C. R., YELLOWLEY C. E., DAVIS B. R., ZHOU Z., CIMBALA J. M., DONAHUE H. J. (1998): 'Differential effect of steady versus oscillatory flow on bone cells', *J. Biomech.*, **31,** pp.969-76

[27] CHARRAS G. T., HORTON M. A. (2002): 'Single cell mechanotransduction and its modulation analyzed by atomic force microscope indentation', *Biophys. J.*, **82,** pp.2970-81

[28] SATO M., THERET D. P., WHEELER L. T., OHSHIMA N., NEREM R. (1990): 'Application of the micropipette technique to the measurement of cultured porcine aortic endothelial cell viscoelastic properties', *J. Biomech. Eng.*, **112,** pp.263-68

[29] JONES W. R., TING-BEALL H. P., LEE G. M., KELLY S. S., HOCHMUTH R., GUILAK F. (1999): 'Alterations in the Young's modulus and volumetric properties of chondrocytes isolated from normal and osteoarthritic human cartilage', *J. Biomech.*, **32,** pp.119-27

[30] COUGHLIN M. F., STAMENOVIC D. (2003): 'A prestressed cable network model of the adherent cell cytoskeleton', *Biophys. J.*, **84,** pp.1328-36

[31] NORIA S., XU F., MCCUE S., JONES M., GOTLIEB A. I., LANGILLE B. L. (2004): 'Assembly and reorientation of stress fibers drives morphological changes to endothelial cells exposed to shear stress', *Am. J. Pathol.*, **164,** pp.1211-23

[32] FROST H. M. (1987): 'Bone "mass" and the "mechanostat": a proposal', *Anat. Rec.*, **219,** pp.1-9

[33] NAUMAN E. A., Ph.D. thesis, University of California, 2000.

[34] ERIKSEN E. F., AXELROD D. W., MELSEN F. (1994): 'Bone Histomorphometry', (Raven Press, New York)

ISSN: 1727-1983