DYNAMICS OF F-ACTIN CYTOSKELETON REORGANIZATION UNDER LOADING-INDUCED OSCILLATORY FLUID FLOW IN MC3T3-E1 PRE-OSTEOBLASTS

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1. INTRODUCTION

Mechanotransduction is the conversion of mechanical stimuli (or signals) into cellular signals. The mechanosensitive signaling pathways of most cells depends on the type of linkages that exist between the cell's cytoskeleton and mechanotransducer molecules [8,9]. Mechanical stresses, distributed through the extracellular matrix, send signals from cell surface adhesion receptors to cellular structures within the cell via the actin cytoskeleton [1]. Differences in the coding of the signals and its target organelle(s) affect the function and development of the cell.

In bone, locomotion and postural control induces dynamic and oscillatory fluid flow in the lacunar-canalicular network of bone tissues [7]. For example, bone tissues in the leg are loaded during the midstance phase of the gait cycle. This causes a deformation in the mineralized matrix, inducing a heterogeneous pressure gradient causing fluid in the lacunar-canalicular network to flow in one direction [2,4,6,7]. When the leg is unloaded during the swing phase of gait, the pressure gradient reverses causing the fluid flow to reverse. This change in direction of flow makes the fluid flow in bone oscillatory in nature.

Experiments done on bone cells in vitro have determined that fluid shear stress is an important signal in mechanotransduction. Reich and Frangos (1991) found that a shear stress of 24 dyn/cm^2 increased inositol triphosphate levels in osteoblast bone cells dramatically for up to 2 hours. When the flow was removed, IP3 levels gradually returned to basal levels. In the same study, fluid shear stress was also found to increase the rate of prostaglandin E2 synthesis by 20-fold at 24 dyn/cm^2. Fredrick et al. (1998) discovered that fluid shear stress increased the expression of COX-2 enzyme and the transcription factor cFos. This phenomenon was induced by a mechanism that involves the reorganization of the actin cytoskeleton under fluid shear. Disruption of the actin cyctoskeleton inhibited the expression of COX-2. However, these studies were all performed with steady fluid flow. In light of recent evidence that fluid flow in bones is dynamic and oscillatory in nature, the effect of oscillating flow on signal transduction must be explored. In this study, we will investigate the effect of applying a loading-induced dynamic and oscillatory fluid flow on the actin cytoskeleton of MC3T3-E1 osteoblastic cells.

2. MATERIAL AND METHODS

Cell Culture- MC3T3-E1 cells, a mouse pre-osteoblast cell line, were cultured in a-MEM media containing 0.7% fetal bovine serum and 1% penicillin-streptomycin at 37°C in a 5% CO^2 humidified incubator. Cells were grown on normal glass and FlexCell? Flex I? (Hillsborough, NC) slides to 80% confluence.

Fluid Flow- Fluid flow was applied to the cells for a period of 60 minutes in a humidified incubator at 37°C with 5% CO^2. Oscillatory and perfusion fluid flows were applied through a parallel plate flow chamber designed by Frangos et al. (1985) with a fluid volume of 62.25 x 24.12 x 0.26 mm. Loading-induced oscillatory flow was calculated to produce a combination of a 11.26 dyn/cm^2 (maximum) oscillatory flow with a 0.00307 dyn/cm^2 (average) variable-speed perfusion flow. A Gastight? Hamilton syringe (Reno, Nevada) driven by an EnduraTEC ELFTM (Minnetonka, MN) was used to generate the 1Hz sinusoidal oscillatory flow. Perfusion flow, generated by a Control Company 3384 pump (Friendswood, TX), provided the cells with fresh media during the length of the experiment. A negative control was conducted by flowing the cells with perfusion flow only.

Positive control experiments were observed by applying a high 11.79 dyn/cm^2 steady laminar flow to the cells (Chen, 2000) with the Flexcell Streamer^{TM} (Hillsborough, NC). The StreamerTM is a six-chamber parallel-plate laminar flow system which holds 75 x 25 x 1 mm culture slides. A Masterflex? pump (Vernon Hills, IL) was used to drive a steady flow into the StreamerTM.

Fluorescence Microscopy- After fluid flow, MC3T3-E1 cells were rinsed twice using PBS and fixed in 3.7% formaldehyde in PBS for 10 minutes. For immunofluorescence microscopy of F-actin, cells were incubated with Molecular Probes Alexa Fluor 488 (FITC) phalloidin (Eugene, OR) for 20 minutes. A glass coverslip was mounted on the slide before observation was made through an Nikon

Eclipse TE300 microscope (Melville, NY) using 10x, 20x, and 40x objectives. Universal Imaging MetaFluor 5.0 software (Downingtown, PA) was utilized to capture still images of the cells immediately after staining.

3. RESULTS

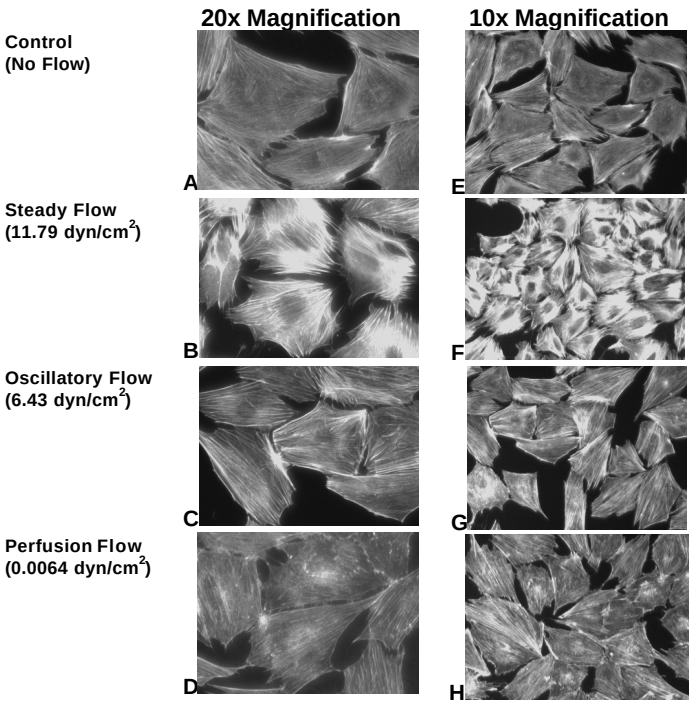

Figure 1. F-actin response of MC3T3-E1 osteoblasts to high unidirectional steady, low unidirectional variable, and loading-induced oscillatory flow for 60 minutes.

Application of loading-induced (oscillatory plus perfusion) flow did not induce the development of F-actin stress fibers in MC3T3-E1 cells. Microscope images in Figure 1 were selected based on the criteria that the cells form a monolayer; with each cell having little contact with surrounding cells. As seen in the experimental control (Fig. 1A and 1E), without fluid shear F-actin fibers were sparsely dispersed in the form of thin strands and were randomly oriented.

Development of thick actin stress fibers was apparent in the high steady flow slide 3B and 3F. Actin stress fibers were orientated parallel to the long axis of the cell and were only present around the nucleus of the cells. This is consistent with previous studies (Pavalko et al., 1998). Furthermore, MC3T3-E1 cells subjected to high fluid shear (steady flow) were smaller in size with irregular-shaped cell membranes when compared with control cells.

Comparing loading-induced (Fig. 1C and 1G) with perfusion flow (Fig. 1D and 1H) slide yielded no apparent differences in the thickness, density, and location of the actin fibers. Similar to the experimental control, actin fibers in both slides were thin in diameter and low in density. There was no evidence of actin stress fiber formation in the physiological and perfusion flow slides. Very few slightly thicker actin fibers were observed at the edge of cell membranes in the physiological flow slide. Cells in all three slides were similar in shape and size.

For all slides in Figure 1, fluid flow was applied horizontally. No observable relationship was found between the direction of fluid flow and the orientation of actin fibers. However, actin fibers tend to combine at the edge of cells when subjected to oscillatory and low fluid flows (loading-induced and perfusion).

4. DISCUSSION

The effect of applying a loading-induced fluid flow on the actin cytoskeleton of MC3T3-E1 osteoblastic cells was investigated for the first time by applying a high oscillatory flow in combination with a low variable perfusion flow. A comparison between MC3T3s that were not subjected to flow (control, Fig. 1A and 1B) with ones subjected to the perfusion flow (Fig 1D and 1E) confirmed that the low variable-speed flow did not affect the results of the loading-induced flow experiment. Although the same magnitude of shear stress was applied through the loading-induced and high steady flow experiments, only the high steady flow caused the reorganization of thin actin filaments into thick actin stress fibers. One possible explanation for this observation is that the actin filaments in MC3T3 are pretensioned by compressed microtubule struts [11]. During flow reversal, microtubules acts to resist compression to retain the shape of the cell. Between reversals, actin filaments act to resist tension caused by the shear stress on the cell [10].

REFERENCES

1. Burridge, K., Chrzanowska-Wodnicka, M., 1996. Focal adhesions, contractility, and signaling. Annu. Rev. Cell Dev. Biol. 12: 463-519.
2. Cowin, S.C., Weinbaum, S., Zeng, Y., 1995. A case for canaliculi as the anatomical site of strain generated potentials. Journal of Biomechanics 28(11), 1281-1297.
3. Frangos, J.A., Eskins, S.G., McIntire, L.V., Ives, C.L., 1985. Flow effects on prostacyclin production by cultured human endothelial cells. Science 227: 1477-1479.
4. Knothe Tate, M.L., Knothe, U., 2000. An ex vivo model to study transport processes and fluid flow in loaded bone. Journal of Biomechanics 33: 247-254.
5. Pavalko, F.M., Chen, N.X., Turner, C.H., Burr, D.B., Atkinson, S., Hsieh, Y., Qiu, J., Duncan, R.L., 1998. Fluid shear-induced mechanical signaling in MC3T3-E1 osteoblasts requires cytoskeleton-integrin interactions. Am J Physiol Cell Physiol 275, C1591-C1601.
6. Piekarski, K., Munro, M., 1977. Transport mechanism operating between blood supply and osteocytes in long bones. Nature 269: 80-82.
7. Weinbaum, S., Cowin, SC., Zeng, Y., 1994. A Model for the excitation of osteocytes by mechanical loading-induced bone fluid shear stresses. Journal of Biomechanics 27: 339-360.
8. Sukharev, S.I., Blount, P., Martinac, B., Kung, C., 1997. Mechanosensitive channels of Escherichia coli: the MscL gene, protein, and activities. Annu Rev. Physiol. 59: 633-657.
9. Zhang, Y., Hamill, O.P., 2000. On the discrepancy between whole-cell and membrane patch mechanosensitivity in Xenopus oocytes. J. Physiol. 523: 101-115.
10. Ingber, D. E. 1993. Cellular Tensegrity: defining new rules of biological design that govern the cytoskeleton. J. Cell Sci. 104: 613-627.
11. Volokh KY, Vilnay O, Belsky M. 2000. Tensegrity architecture explains linear stiffening and predicts softening of living cells. J Biomech. 33:1543-9.