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Bone marrow stem cells adapt to low-magnitude vibrations by altering their cytoskeleton during quiescence and osteogenesis

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Abstract: Application of mechanical vibrations is anabolic to bone tissue, not only by guiding mature bone cells to increased formation, but also by increasing the osteogenic commitment of progenitor cells. However, the sensitivity and adaptive response of bone marrow stem cells to this loading regimen has not yet been identified. In this study, we subjected mouse bone marrow stem cell line D1-ORL-UVA to daily mechanical vibrations (0.15 g, 90 Hz, 15 min/day) for 7 days, both during quiescence and osteogenic commitment, to identify corresponding ultrastructural adaptations on cellular and molecular levels. During quiescence, mechanical vibrations significantly increased total actin content and actin fiber thickness, as measured by phalloidin staining and fluorescent microscopy. Cellular height also increased, as measured by atomic force microscopy, along with the expression of focal adhesion kinase (PTK2) mRNA levels. During osteogenesis, mechanical vibrations increased the total actin content, actin fiber thickness, and cytoplasmic membrane roughness, with significant increase in Runx2 mRNA levels. These results show that bone marrow stem cells demonstrate similar cytoskeletal adaptations to low-magnitude high-frequency mechanical loads both during quiescence and osteogenesis, potentially becoming more sensitive to additional loads by increased structural stiffness.

Key words: Mesenchymal stem cells, vibrations, in vitro cell culture, mechanical signals, osteogenic commitment, bone, cytoskeleton, biomechanics

1. Introduction

Mechanical signals are essential for the turnover of bone tissue, but a complete picture of how bone cells sense and interpret these mechanical signals still remains elusive (Rubin et al., 2001, 2002). Superposition of mechanical signals onto habitual activity augments bone mass (Snow-Harter et al., 1992), which may mitigate individual bone loss associated with aging, sedentary lifestyles, or space travel (Ozcivici et al., 2010a; Özcivici, 2013). Exogenous mechanical stimuli need not be comparable in magnitude to signals that are regularly experienced during habitual activity to be effective, as extremely low-magnitude (≤0.3 g, 1 g = 9.81 m/s^2) vibratory signals are also associated with anabolism in the bone through the activation of osteoblasts tissue when applied at high frequencies (>30 Hz) (Xie et al., 2006). Furthermore, low-intensity vibrations increase the pool of progenitor cells that reside in the bone marrow and guide their commitment towards osteogenesis (Rubin et al., 2007). Even under strong catabolic stimuli induced by mechanical unloading, daily applications of low-intensity vibrations protect the progenitor cell pool in the bone marrow and enhance osteoblastogenesis and bone recovery when the catabolic stimulus is over (Ozcivici et al., 2010b).

Low-intensity vibrations induce a broad range of cellular and molecular responses when applied to osteoblasts/osteoprogenitors in vitro. The repertoire of responses includes increased proliferation, enhanced release of secondary messengers, induced mRNA expressions for osteogenic markers, and increased mineralization (Rosenberg et al., 2002; Tanaka et al., 2003; Batra et al., 2005; Pre et al., 2011). Notwithstanding the beneficial outcomes observed for bone regeneration, cellular events that modulate responsiveness against low-intensity vibrations are still not well defined. This is in part because such a definition would first require an understanding of the governing force component that acts on the cell during low-intensity mechanical vibrations.

Fluid shear/drag forces acting on the cell membrane may be a potential candidate, as fluid shear is a more potent inducer of osteogenesis compared to substrate deformations (You et al., 2000). Another alternative hypothesis for the effectiveness of vibratory signals is the "oscillatory motion" component of these signals

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that prescribes accelerations on the cell and subcellular structures (Garman et al., 2007a, 2007b; Ozcivici et al., 2007). Recent in vitro evidence suggests that during oscillatory fluid shear applications, a mechanical response is modulated by accelerations occurring during fluid flow rather than drag forces acting on the cellular membrane (Uzer et al., 2012, 2013).

If oscillatory accelerations and resultant motions can modulate mechanotransduction, cellular ultrastructural elements will be an important determinant for the transfer of prescribed motion, since transduction of mechanical signals in cells is mainly dependent on the cytoskeletal network (Ingber, 1997, 2003b; Dahl et al., 2010). Consistent with the idea that cytoskeletal elements are strong determinants of mechanotransductive pathways, chemical blocking of actin polymerization inhibits the response of bone cells to mechanical stimulation (Rosenberg, 2003). Similarly, molecular response to mechanical stimuli is augmented in progenitor cells during osteogenesis, when the treatment is combined with lysophosphatidic acid, an agent that induces rapid actin stress fiber formation (Uzer et al., 2013). Though cytoskeletal elements can guide the mechanical sensitivity of bone-forming cells during low-intensity vibrations, it is not clear whether cytoskeletal elements can adapt to this oscillatory stimulus, similar to adaptations observed in response to fluid shear (Malone et al., 2007; Ponik et al., 2007).

The relationship between transmission of mechanical signals and cytoskeleton becomes more complex with the process of osteogenic commitment, because stem cells increase their global mechanical stiffness during osteogenesis (Darling et al., 2008), a process that is also modulated by alterations in the actin cytoskeletal network (Yourek et al., 2007). It is not clear whether this increase in prestress of cells during osteogenic commitment is required for a marrow stem cell to respond to mechanical loads, and whether quiescent (noncommitting) stem cells can also adapt to vibratory stimulus (Wang and Suo, 2005; Hu and Wang, 2006). To address this question, we subjected D1-ORL-UVA mouse bone marrow stem cells to daily vertical mechanical vibrations in vitro, with or without chemical induction of osteogenesis, for 7 days. We measured the adaptation of cytoskeletal elements of bone marrow progenitor cells to mechanical stimulus by using fluorescence labeling, atomic force microscopy, and gene expression analysis.

2. Materials and methods

2.1. Cell culture

Mouse bone marrow stem cell line D1-ORL-UVA (American Type Culture Collection, USA) was used in all experiments. D1-ORL-UVA cells were grown and maintained in Dulbecco's modified Eagle's medium (Thermo Scientific HyClone, USA) with high glucose, L-glutamine, and sodium bicarbonate and supplemented with 10% fetal bovine serum (Biological Industries, Israel) and 1% penicillin/streptomycin (Biological Industries), as instructed by the vendor. For all experiments, D1-ORL-UVA cells were used between passages 6 and 12. Osteogenic induction was achieved by adding 1000 μg/mL ascorbic acid (Sigma, USA) and 10 mmol β-glycerol phosphate (Sigma) to the growth medium, which induced clear mineralized nodules by alizarin stain in 2 weeks of cell culture (Figure 1). For all experiments except atomic force microscopy (AFM), D1-ORL-UVA cells were plated at a density of 1×10^4 cells/well in 6-well plates (Corning, USA) on a sterilized 22 × 22 cm^2 glass cover slide and maintained in the growth medium at 37 °C and 5% CO_2. Cells were allowed to adhere to the cover plate for 2 days; after 2 days, growth media were either refreshed (for quiescence) or changed with osteogenic media (for osteogenesis). Culture media were changed every 3 days and all experiments were terminated on day 9. For AFM experiments, cells were grown on sterilized glass slides with a diameter of 1 cm.

2.2. Mechanical stimulation

In an effort to test the effects of low-magnitude vibrations on adult bone marrow stem cells with or without osteogenic

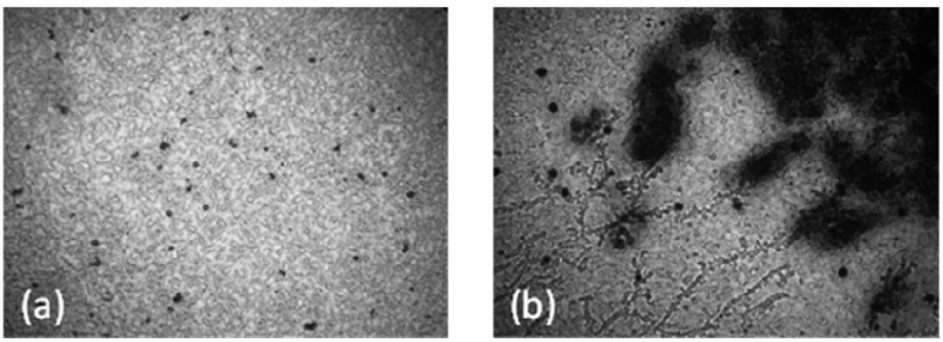

Figure 1. Alizarin red staining of D1-ORL-UVA cells at 16 days of experimentation. No nodules were observed in quiescent cells in growth media **(a)**, while clearly mineralized nodules of calcified matrix were present in cultures containing osteogenic media **(b)**.

commitment, cells were either subjected to 90 Hz, 0.15 g vibrations for 7 days (15 min/day) under room conditions or received a sham treatment to eliminate the effects of ambient conditions. The mechanical signal used for this study was shown to be anabolic for bone and bone marrow MSCs in vivo (Ozcivici et al., 2010b). Proper sinusoidal signal was provided by a custom-made platform, and the mechanical signal quality was continuously controlled with a real-time accelerometer (K-Beam, Kistler, USA). Measurements were monitored by LabVIEW 2010 Signal Express (National Instruments, USA). D1-ORL-UVA cells that were kept in the growth media and received daily mechanical loading were reported as the growth vibration (GV) group, whereas D1-ORL-UVA cells that received daily sham loading were reported as the growth control (GC). Similarly, D1-ORL-UVA cells that were cultured in osteogenic media that received daily loading were reported as the osteogenic vibration (OV) group, while osteogenic D1-ORL-UVA cells that received sham loading were reported as the osteogenic control (OC).

2.3. Cell growth and viability

On day 9, cover glasses were removed to a new 6-well plate and cells were trypsinized. The number of cells in the experimental and control groups was quantified using trypan blue dye exclusion method, where cells were diluted with 0.4% trypan blue dye (GIBCO, Invitrogen, USA) in a 1:1 ratio and counted with a Neubauer hemocytometer. Cell viability was analyzed via MTT assay, in which cells were incubated with 0.5 mg/mL MTT (Amresco LLC, USA) for 4 h. The incubation tetrazolium salts were subsequently dissolved in 600 µL of DMSO for 2 min and colorimetric measurements were done at 570 nm with a background subtraction at 650 nm.

2.4. Immunostaining and fluorescent microscopy

Cells were washed in phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde (PFA) for 20 min. PFA was triple-washed with PBS, followed by membrane permeabilization with 0.1% Triton X/PBS for 15 min. The agent was blocked with 3% bovine serum albumin in 0.1% Triton X/PBS for 30 min. Cells were then incubated in the dark with phalloidin dye (Invitrogen) for 30 min for the imaging of actin filaments. After gentle washing with PBS, cells were incubated in DAPI solution for visualization of nuclei. Images were acquired with an inverted microscope and fluorescent attachment (CKX71, Olympus, Japan). Micrographs of actin cytoskeleton were acquired at 647 nm and nuclear structures were imaged at 350 nm for all groups with similar exposure times (Figure 2). A minimum of 10 sample images were used for signal intensity and fiber thickness analysis per condition from 3 different experiments. Images were analyzed using ImageJ software.

2.5. Atomic force microscopy

The Digital Instruments MMSPM Nanoscope IV (Bruker, USA) was used to get AFM images. Cells were washed with ultrapure water and dried in ambient conditions for 10 min. Cells were then probed with a soft silicon cantilever with semiangle of 35° and 8 N/m spring constant. Locations of cells were detected using an optical microscope (Nikon, Japan), and the cantilever tip was conveniently adjusted above the observed cells. Cells were scanned at 1001 Hz for 512 samples (Figures 3a and 3b). Cells were analyzed for average surface height, roughness of cytoplasmic regions, and physical characteristics of nucleus using AFM image processing software Gwyddion (Figure 3c). A minimum of 10 cell scans were used for each group that was collected from at least 3 separate experiments.

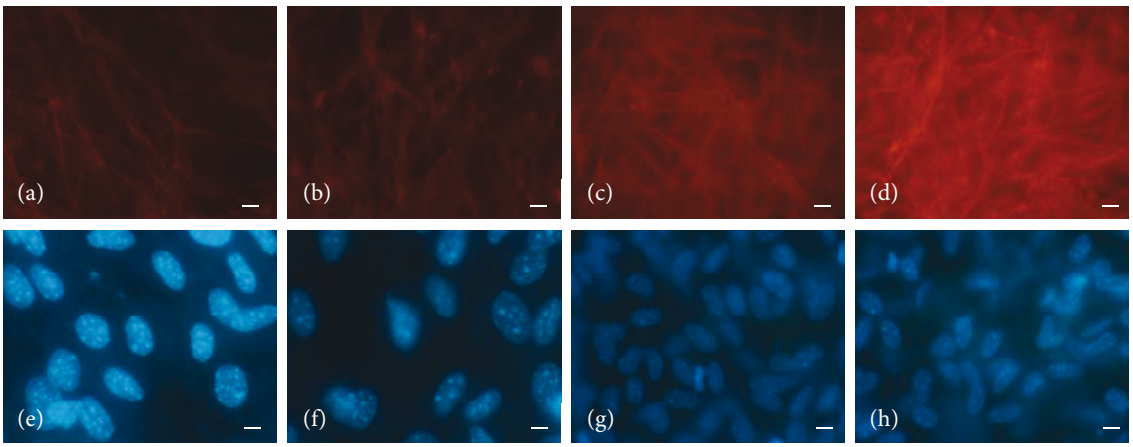

Figure 2. Representative fluorescent micrographs for phalloidin (red) and DAPI (blue) stains from **(a, e)** GC cells, **(b, f)** GV cells, **(c, g)** OC cells, and **(d, h)** OV cells. GC: Growth control, GV: growth vibration, OC: osteogenic control, OV: osteogenic vibration. Scale bars = 10 µm.

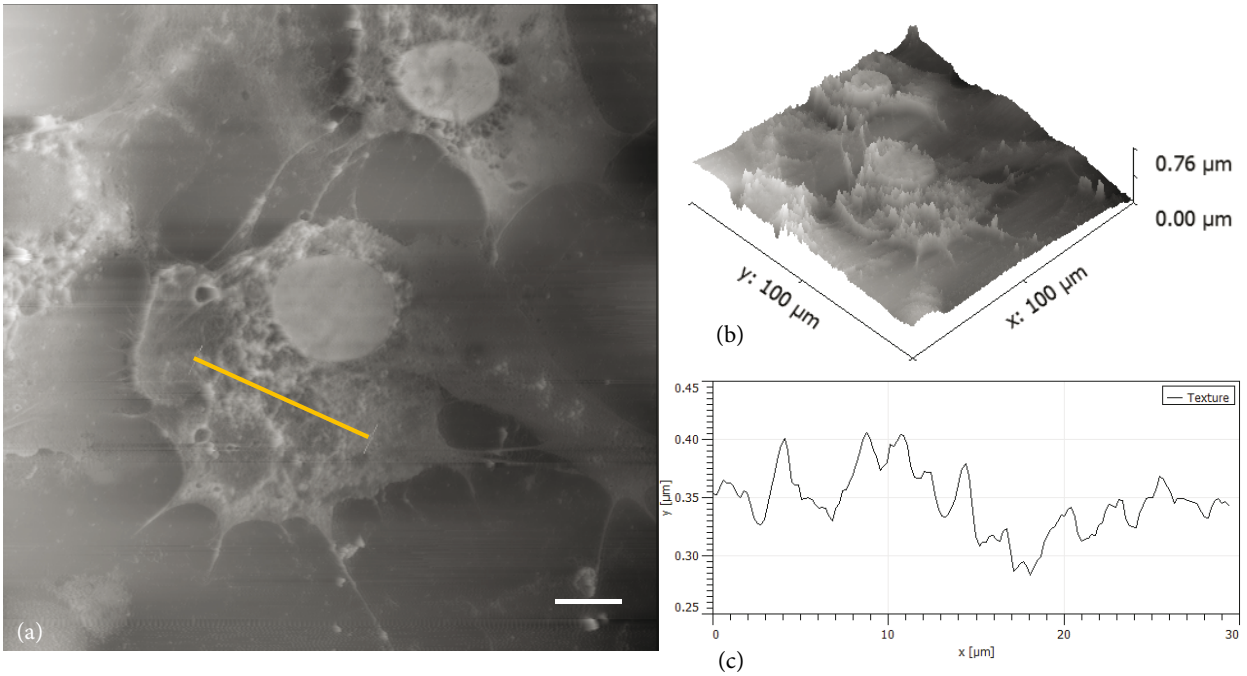

Figure 3. A representative section for AFM scans of bone marrow stem cells. **(a)** Two-dimensional height data from the mapped region, **(b)** 3D representation of the mapped region, and **(c)** 2D plot of a representative height profile (shown as orange in **a**) obtained from nonnuclear cytoplasmic areas that were used for the calculation of average height and roughness of individual cells. Scale bar = 10 µm.

2.6. Gene expression analysis

Cells were lysed and total mRNA was isolated using the PureLink RNA mini kit (Invitrogen, Lot 1267010). After verification of purity and determination of concentration by the ND-1000 NanoDrop (Thermo Scientific), 2-step real-time polymerase chain reaction (PCR) was performed. For reverse-transcription (RT) reaction, the RevertAid first-strand cDNA synthesis kit (Thermo Scientific) was used with 1000 ng of template RNA. cDNA samples of 7.5 µL were loaded with 12 µL of SYBR Green (Thermo Scientific), 2.5 µL of forward and reverse primers of osteogenic markers, and cytoskeletal molecules (Table 1) for quantitative RT-PCR (Bio-Rad, USA), where GAPDH was used as the house-keeping molecule. All groups used 3 or 4 samples for gene expression analysis.

2.7. Statistical analysis

All results are expressed as mean ± standard deviation. Group comparisons were done by using unpaired t-tests between control and vibration groups, in which the threshold for statistical significance was set to 5%. Microscopy samples (both fluorescence and atomic force) were maintained and measured on the same days for growth and osteogenic groups; therefore, comparisons were only made within groups. Samples that were used for gene expression analysis were maintained and measured together for all groups.

Table 1. Primers designed for the gene expression analysis of osteogenic markers (Runx2, OCN) and cytoskeletal elements (β-actin, desmin, vimentin, β-tubulin, PTK2) for D1-ORL-UVA mouse mesenchymal stem cells. GAPDH was used as the house-keeping molecule for all groups.

Gene	Direction	Sequence
Runx2	F	TCC CTG AAC TCT GCA CCA AGT
	R	TTC CGT CAG CGT CAA CAC CAT
OCN	F	CTG ACA AAG CCT TCA TGT CCA A
	R	GCG CCG GAG TCT GTT CAC TA
β-Actin	F	CTT CTT TGC AGC TCC TTC GTT
	R	TTC TGA CCC ATT CCC ACC A
Desmin	F	GTG AAG ATG GCC TTG GAT GT
	R	GTA GCC TCG CTG ACA ACC TC
Vimentin	F	ACG GTT GAG ACC AGA GAT GG
	R	CGT CTT TTG GGG TGT CAG TT
β-Tubulin	F	GAT GGG CAA CTG TAC CTG ACT G
	R	CTG GGC TCC TCT TGG AAT G
PTK2	F	TTG GAC CTG GCA TCT TTG AT
	R	AGA ACA TTC CGA GCA GCA AT
GAPDH	F	GAC ATG CCG CCT GGA GAA AC
	R	AGC CCA GGA TGC CCT TTA GT

3. Results

3.1. Cell growth and viability

In order to compare the effect of vibration on cell growth, cells were counted with the trypan blue dye exclusion method (Table 2). The number of cells in the GV group had increased by 13% (P = 0.05) at the end of the 7-day procedure compared to GC. No significant difference (P = 0.32) was observed between OC and OV cells. MTT assays showed that cells belonging to the GV group demonstrated a small but significant (1%, P = 0.05) increase in signal compared to the GC group. Similarly, OV cells also showed 3% (P < 0.01) increase in MTT signal compared to OC cells (Table 2).

Table 2. Number of cells as counted by trypan blue dye exclusion and cell viability by MTT assay. Results are presented mean ± SD. *: P < 0.05 between growth control and vibration groups, †: P < 0.05 between growth control and vibration groups. GC: Growth control, GV: growth vibration, OC: osteogenic control, OV: osteogenic vibration.

Group	Number of cells [× 10^5/mL]	Cell viability [a.u.]
GC	0.98 ± 0.17	3.46 ± 0.05
GV	1.10 ± 0.25*	3.51 ± 0.07*
OC	1.54 ± 0.61	3.40 ± 0.06
OV	1.72 ± 0.36	3.49 ± 0.06†

3.2. Immunostaining and fluorescent microscopy

In an effort to delineate cytoskeletal differences between groups, the histograms of fluorescence intensity distributions for cells treated with phalloidin (Figure 4a) were analyzed for mean intensity values as well as mean intensity values normalized to the number of counted cell nuclei. Images obtained from the GV group were similar (P = 0.37) in mean phalloidin signal intensity compared to the GC group. Once normalized to the number of cells counted with DAPI stains within corresponding images, GV cells showed 20% (P = 0.04) more mean intensity per cell compared to GC cells (Figures 4b and 4c). Furthermore, actin fibers in the GV group were 46% (P = 0.02) thicker than the fibers observed in control cells (Figure 4d).

Intensity histograms that showed actin cytoskeletons of D1-ORL-UVA cells in osteogenic conditions (Figure 5a) were also analyzed after 1 week of culture. Signal intensity acquired from OV group images had 21% (P < 0.01) more mean intensity and 25% (P < 0.01) more mean intensity per cell compared to the intensity levels of the OC group (Figures 5b and 5c). Moreover, actin fibers in OV cells were 14% (P = 0.02) thicker than the fibers observed in OC cells (Figure 5d).

3.3. Atomic force microscopy

In order to characterize cortical cytoskeletons via surface roughness of cells, AFM scans for whole cells were performed. Average cytoplasm surface values of the GV

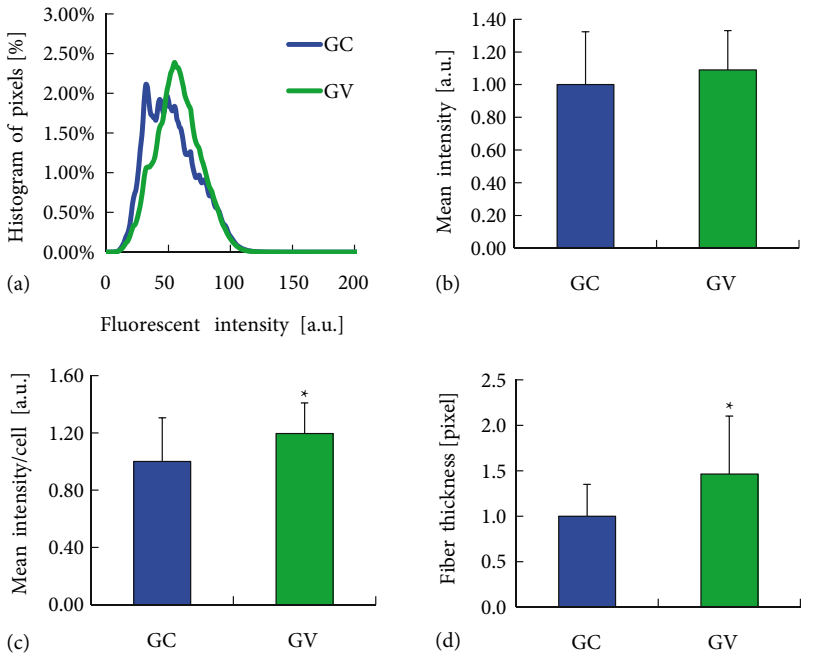

Figure 4. Immunostaining and fluorescent microscopy results of bone marrow stem cells that were maintained in quiescence. **(a)** Average distributions of phalloidin intensity, **(b)** mean intensity, **(c)** mean intensity normalized by number of cells counted by DAPI stains, and **(d)** average fiber thickness. Results are presented mean ± SD. *: P < 0.05 between growth control and vibration groups. GC: Growth control, GV: growth vibration.

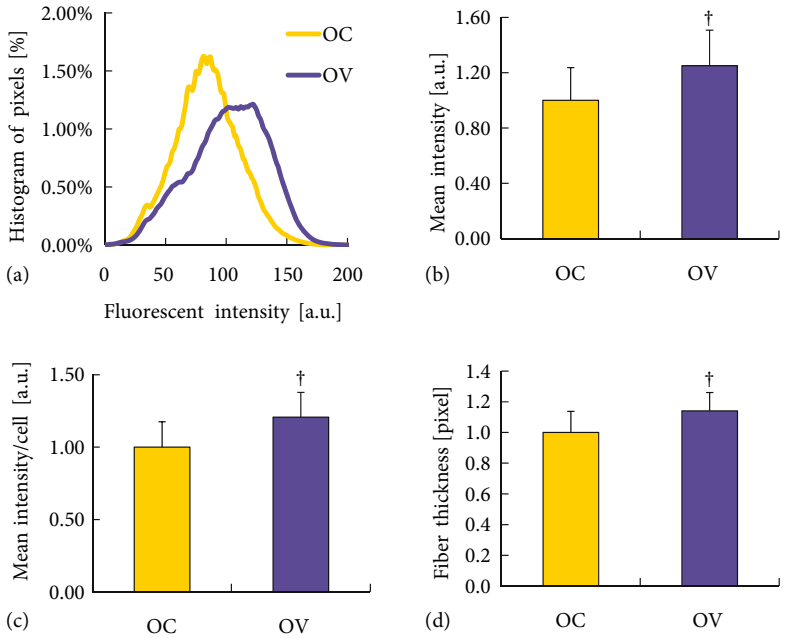

Figure 5. Immunostaining and fluorescent microscopy results of bone marrow stem cells that were chemically induced for osteogenesis. **(a)** Average distributions of phalloidin intensity, **(b)** mean intensity, **(c)** mean intensity normalized by number of cells counted by DAPI stains, and **(d)** average fiber thickness. Results are presented mean ± SD. †: $P < 0.05$ between growth control and vibration groups. OC: Osteogenic control, OV: osteogenic vibration.

group were 98% ($P < 0.01$) higher compared to GC cells (Figure 6a). Similarly, nuclear height for GV cells was 78% ($P < 0.01$) higher than the average height of GC cells (Table 3). No significant differences were observed between OC and OV cells ($P = 0.72$) regarding average cytoplasm height, but the nuclear height of OV cells was 33% ($P = 0.04$) higher compared to OC cells (Figure 6b; Table 2). Membrane roughness measured over the cytoplasm surface of GV cells was similar ($P = 0.10$) to the GC group (Figure 6c), but OV cells were 22% ($P = 0.04$) rougher than OC cells (Figure 6d).

Table 3. Nuclear height (nm) of cells as determined by AFM scans. Results are presented as mean ± SD. *: $P < 0.05$ between growth control and vibration groups, †: $P < 0.05$ between growth control and vibration groups. GC: Growth control, GV: growth vibration, OC: osteogenic control, OV: osteogenic vibration.

Group	Nucleus height [nm]
GC	391 ± 103
GV	694 ± 146*
OC	480 ± 153
OV	638 ± 198†

3.4. Gene expression analysis

Gene expression patterns, as tested with real time RT-PCR normalized to GC data, confirmed that the process of osteogenesis showed a significant increase in osteogenic markers such as Runx2 and osteocalcin (OCN), without any evidence related to the effect of vibrations (Figure 7). Among ultrastructural elements, β-actin showed close to 2-fold increase ($P = 0.01$) during osteogenesis compared to GC cells, again without any potential effect from mechanical vibrations. Osteogenesis also showed more than 20-fold increase in the expression of PTK2 (focal adhesion kinase) for osteogenic cells ($P < 0.01$) compared to GC cells. Interestingly, the GV group also showed a similar increase ($P < 0.01$) in the expression of PTK2 compared to the GC group, with similar expression levels to osteogenic groups. Vibrations significantly ($P < 0.01$) increased Runx2 mRNA levels in D1-ORL-UVA cells during osteogenesis.

4. Discussion

In this study, we identified the early effects of low-magnitude high-frequency mechanical vibrations on the ultrastructural properties of bone marrow mesenchymal stem cells in vitro. Briefly, the daily application of mechanical vibrations (0.15 g, 90 Hz) increased the density

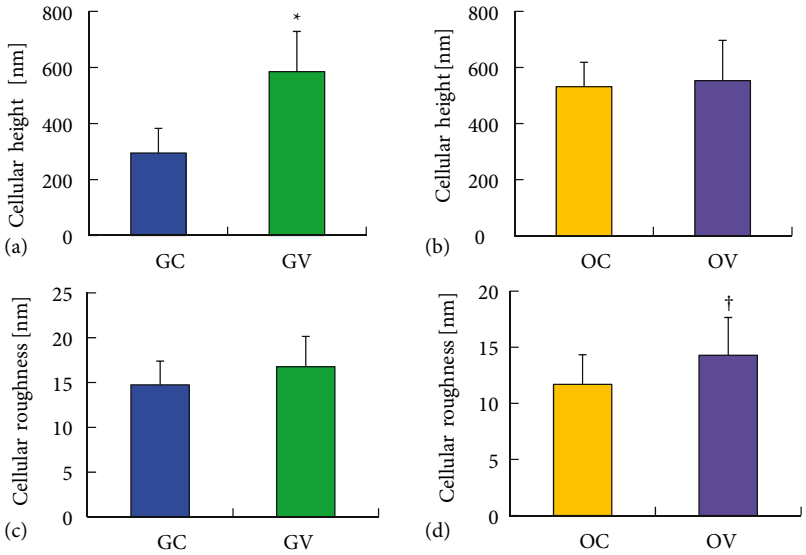

Figure 6. AFM measurement results of cell surface height for **(a)** quiescent and **(b)** osteogenic cells, and cell surface roughness for **(c)** quiescent and **(d)** osteogenic cells. Results are presented as mean ± SD. *: $P < 0.05$ between growth control and vibration groups, †: $P < 0.05$ between growth control and vibration groups. GC: Growth control, GV: growth vibration, OC: osteogenic control, OV: osteogenic vibration.

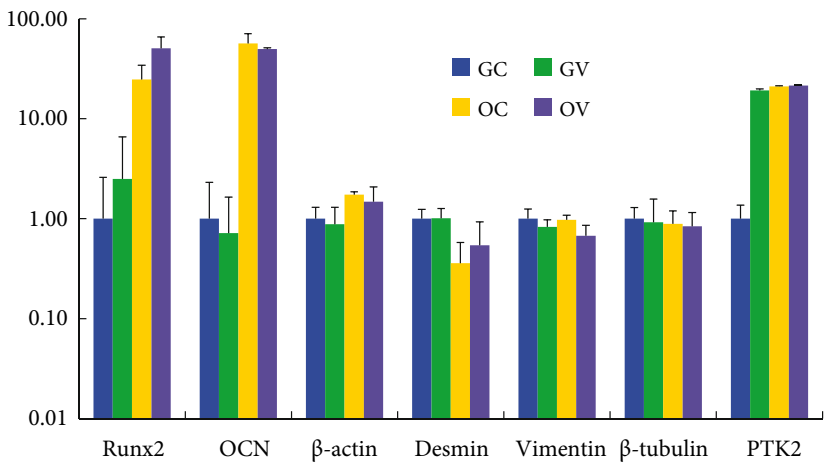

Figure 7. Real time RT-PCR results for selected molecular markers. Expression levels were first normalized to GAPDH expression for corresponding groups and then to GC results. Results are presented as mean ± SD. GC: Growth control, GV: growth vibration, OC: osteogenic control, OV: osteogenic vibration.

and thickness of actin fibers in stem cells. Vibrations also affected cellular morphology by increasing the cellular height of stem cells without osteogenic commitment and membrane roughness of stem cells with osteogenic commitment. Results also indicated an increase in proliferation for bone marrow stem cells after 1 week, both during quiescence and osteogenic commitment. Low-intensity vibrations also increased molecular expression of focal adhesion kinase in quiescent stem cells, potentially changing cell-to-cell and cell-to-substrate interactions. Overall, results indicated a differential effect for mechanical vibrations on stem cells based on their lineage commitment.

Several limitations should be acknowledged regarding the interpretation of our findings. Our fluorescent image readings of phalloidin intensities were recorded with an inverted microscope without any confocal capabilities; therefore, the results were an indicator of the ultrastructure

at the focus of the objective rather than a potential segmentation for the entire depth of the cells (Malone et al., 2007). Another limitation of our experimental design concerns the nature of the AFM methodology. We were not able to achieve the scanning of live cells in "fluid cell apparatus", which is a physiologically more relevant test for cellular membranes (Pesen and Hoh, 2005). Therefore, our results regarding cell morphology and roughness should be treated as relative rather than absolute (Qian et al., 2010).

Mechanical vibrations were shown to be anabolic for osteoprogenitor cell pools in vitro, whether the application of stimulus was vertical (Kim et al., 2012) or horizontal (Uzer et al., 2013). Our results were consistent with the increase in cell proliferation for quiescent stem cells, but we did not observe an increase in cell numbers of stem cells during osteogenic commitment, even though our results suggested increased viability. The reason for this discrepancy may be explained by the different natures of the 2 methods. Cell counting requires trypsinization and removal of the cells from the substrate layer. This process is not very efficient with osteogenic cells because a heavy layer of collagen matrix and the initiation of calcified depots trap some of the cells. MTT assay, on the other hand, is an in situ colorimetric experiment that requires little effort for the extraction of the dye. Overall, we think that the signal used in this study affected the proliferation of both quiescent cells and cells in osteogenic commitment.

Adaptive response of cells to exogenous mechanical signals involves critical alterations in their ultrastructural properties. Fibroblasts, for example, readily adapt to cyclic shear by reorientation and reinforcement of actin stress fibers (Yoshigi et al., 2005). Similarly, bone-forming osteoblasts adapt to unidirectional (Norvell et al., 2004) or oscillatory (Ponik et al., 2007) flow conditions by forming new stress fibers. Bone marrow stem cells also respond to biaxial substrate deformations by altering the cytoskeletal network (Sen et al., 2013). Our results indicate that marrow stem cells can also increase and reinforce their cytoskeleton in response to daily application of vertical oscillatory motions. This response was observed in both the quiescent and the osteogenic state, suggesting that marrow stem cells change their subcellular confirmation to become stiffer and probably more sensitive to additional loads (Ingber, 2003a).

Similar to the responsiveness to mechanical loads, cytoskeletal adaptations that occur during osteogenic commitment of stem cells involve the reorganization of stress fibers and the increase of membrane roughness, which conspire towards increased cellular stiffness (Chen et al., 2010). Cellular morphology and membrane roughness of bone-inducing cells were shown to be associated with the presence of mechanical loads, as the removal of gravitational loads with high magnitude of environmental gradient (0 g using magnetic levitation) reduced the height and the roughness of these cells (Qian et al., 2010). Our results suggest that daily addition of oscillatory loads increases the roughness of the cellular membrane for marrow stem cells that commit to osteogenesis. At this point, it is not clear whether this change in roughness is caused by increased mineralization at the surface (Chen et al., 2010) or altered cytoskeletal properties (Yourek et al., 2007) as a result of vibrations.

Though whole-body vibratory signals are applied to the entirety of the organism in a vertical manner (Rubin et al., 2004; Gilsanz et al., 2006), the best loading condition to simulate these signals in in vitro modeling has not been optimized yet. Horizontal rather than vertical loading in vitro (Uzer et al., 2012) can generate fluid shear forces on cells that are physiologically relevant for bone-lining osteoblasts (Dickerson et al., 2008; Coughlin and Niebur, 2012). However, recent evidence showed that accelerations, not fluid shear, are the key determinants for cellular adaptation to oscillatory loads (Uzer et al., 2013). While it is not clear whether horizontal accelerations have similar effects as those achieved by vertical loading, the application of vertical signals may be physiologically more relevant in modeling stem cell response, as these primitive osteoprogenitor cells usually do not reside on the bone surface (Mendez-Ferrer et al., 2010).

In summary, daily high-frequency low-magnitude vibrations affected the cytoskeleton and the morphology of bone marrow stem cells during osteogenesis and quiescence. The increased amount and thickness of actin fibers indicated that stem cells became more sensitive to mechanical loads and that these loads can be transmitted more easily within the cell (Hu et al., 2005; Wang and Suo, 2005; Hu and Wang, 2006). Increased stiffness of cells may further indicate that they may act as stress-absorbing elements and divert some of the loads that are normally carried by the extracellular matrix onto themselves, similar to the "stress shielding" observed in orthopedic implants (Cristofolini, 1997; Bush et al., 2006). Improved understanding of the reciprocal relationship between bone marrow stem cells and mechanical loads may help clinical efforts for bone regeneration through optimization of the required amplitude and duration of the applied signals.

Acknowledgments

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