Published in final edited form as:

Microgravity Sci Technol. 2009 November ; 21(4): 311–318. doi:10.1007/s12217-008-9092-6.

Magnetic Levitation of MC3T3 Osteoblast Cells as a Ground-Based Simulation of Microgravity

Bruce E. Hammer,

Department of Radiology, University of Minnesota, 420 Delaware St., MMC 292, Minneapolis, MN 55455, USA, URL: www.ciamr.umn.edu

Louis S. Kidder,

Department of Radiology, University of Minnesota, 420 Delaware St., MMC 292, Minneapolis, MN 55455, USA, URL: www.ciamr.umn.edu

Philip C. Williams, and

Department of Radiology, University of Minnesota, 420 Delaware St., MMC 292, Minneapolis, MN 55455, USA, URL: www.ciamr.umn.edu

Wayne Wenzhong Xu

Supercomputing Institute for Advanced Computational Research, University of Minnesota, 117 Pleasant St., Minneapolis, MN 55455, USA

Bruce E. Hammer: hammer@umn.edu

Abstract

Diamagnetic samples placed in a strong magnetic field and a magnetic field gradient experience a magnetic force. Stable magnetic levitation occurs when the magnetic force exactly counter balances the gravitational force. Under this condition, a diamagnetic sample is in a simulated microgravity environment. The purpose of this study is to explore if MC3T3-E1 osteoblastic cells can be grown in magnetically simulated hypo-g and hyper-g environments and determine if gene expression is differentially expressed under these conditions. The murine calvarial osteoblastic cell line, MC3T3-E1, grown on Cytodex-3 beads, were subjected to a net gravitational force of 0, 1 and 2 g in a 17 T superconducting magnet for 2 days. Microarray analysis of these cells indicated that gravitational stress leads to up and down regulation of hundreds of genes. The methodology of sustaining long-term magnetic levitation of biological systems are discussed.

Keywords

Magnetic levitation; MC3T3; Osteoblastic cell; Microgravity

Introduction

An understanding of the physical and biological effects of reduced gravitation is necessary to conduct long-term operations in earth orbit, deep space or environments where the gravitational force is less than 1 g. The logistics of carrying-out these kinds of studies in non-terrestrial environments is technologically and economically formidable. On earth, it is possible to simulate orbital freefall for a few minutes through parabolic flight or for a few seconds using drop towers. Both of these methods provide the desired environment for a short time and are accompanied by large changes in acceleration which can confound the

experimental results. A method commonly used to simulate a reduced gravity environment for cellular systems is the 2D or 3D rotating clinostat which randomizes a cell's orientation with respect to the gravitation field (Sarkar et al. 2000; Yuge et al. 2003). In this environment cells are exposed to fluid shear (Pavalko et al. 1998; Jacobs et al. 1998) which can obfuscate if a cell is responding to a randomized gravity vector or fluid effects. The same is true for utilizing rotating wall vessels to simulate microgravity (Kaysen et al. 1999). Another method to simulate orbital freefall hypo gravity and hyper gravity is to generate a magnetic force on the object of interest. Placing a biological (diamagnetic) object in a strong magnetic field and a strong magnetic field gradient creates a magnetic force on the system (Ueno and Iwasaka 1994; Brooks et al. 2000). Magnetic levitation occurs when the magnetic force exactly counterbalances the gravitational force. When the magnetic forces do not cancel it is possible to have a net force less than 1 g or greater than 1 g. Magnetic levitation is capable of sustaining continuous levitation for weeks at a time and has the capability of serving as a ground-based method to modify net gravitational forces experienced by biological systems (Guevorkian and Valles 2004).

Concept of Magnetic Levitation for Biological Systems

All materials experience a force when placed in a region of magnetic field and field gradient (Beaugnon and Tournier 1991; Berry and Geim 1997). The magnitude of this force depends upon the magnetic susceptibility of the material which may vary over a wide range depending on the type of material. Ferromagnetic materials have large susceptibilities and can generate large forces even in modest fields and gradients. Force interactions between ferromagnetic materials and magnetic fields are evident in everyday life and even more so when working with high magnetic fields. In particular, superconducting magnets, because of their compact size, tend to have high field gradients with associated high forces. Depending on the field and configuration of the magnet, these forces can be so high as to be hazardous and special operating procedures need to be in place to ensure that ferromagnetic items are kept to a safe distance. For the weakly magnetic materials (dia- and paramagnets) the forces involved are much smaller and not obvious in everyday experience. Very high magnetic fields and magnetic field gradients are required to produce measurable forces.

The physical principle behind magnetic levitation is that all diamagnetic objects magnetically polarize when immersed in a strong magnetic field. In the presence of a strong magnetic field gradient, parallel to the polarizing field, the diamagnetic sample experiences a magnetic force. Orienting this force opposite to the gravitational force leads to no net force on the object; thus, levitation occurs. The force per unit mass is

$$f = \frac{\chi_\rho}{2} B_z \frac{\partial B_z}{\partial z} - g$$

where χ_ρ is specific magnetic susceptibility, (m^3/kg), B_z is the field, $\partial B_z / \partial z$ is the field gradient (T/m) and g is the constant for gravitational acceleration (m/s^2). The field-gradient product is defined as $B_z \times \partial B_z / \partial z$. Note that χ_ρ is a molecular property and the value of the field-gradient product necessary for levitation is *independent* of mass. Magnetic levitation requires a very strong magnetic field to induce magnetism in a diamagnetic sample and a very strong field gradient to generate a magnetic force on the sample (Valles et al. 2002). For levitation to occur in most diamagnetic materials we must satisfy the relation $1,300 \text{ T}^2 \text{ m} < (B_z) \times (\partial B_z / \partial z) < 1,600 \text{ T}^2 \text{ m}$, e.g. for $1,400 \text{ T}^2/\text{m}$ either $B_z = 10 \text{ T}$, $\partial B_z / \partial z = 140 \text{ T/m}$ or $B_z = 12.5 \text{ T}$, $\partial B_z / \partial z = 125 \text{ T/m}$ satisfies the criteria for levitation.

Magnetic levitation eliminates all stresses and pressures from gravitational forces and most closely mimics orbital freefall. This phenomenon is different from objects experiencing neutral buoyancy in a gas or liquid fluid (Valles et al. 1997). Adjusting the polarizing field or gradient field can vary the magnetic force. Magnetic levitation acts on each molecule of the system and is not a surface phenomenon. That is why magnetic levitation can be described as a surrogate for orbital freefall. In a magnet, changing the physical location of a sample changes the magnetic force on the sample (Fig. 1). This opens the possibility of varying the net force on a sample from zero to two or more g's for ground-based experiments. In a recent study by Coleman et al. (2007), it was found that growing *Saccharomyces cerevisiae* in hypo-g and hyper-g magnetically simulated environments resulted in measurable changes in gene expression, cell growth and cell cycle timing.

Magnet Description

Magnetic levitation of diamagnetic samples in a room temperature environment was first demonstrated by Beaunon and Tournier (1991) using a resistive 14 T Bitter magnet with a 32 mm warm bore. Beaunon magnetically levitated diamagnetic liquids (water, alcohol) and solids (bismuth, antimony) in test tubes. Berry and Geim (1997) reported magnetic levitation of diamagnetic samples at the University of Nijmegen in The Netherlands. In a Bitter magnet at 13 T with a 100 T/m gradient they were able to levitate water, acorns and living frogs within the 35 mm warm bore. Valles et al. (1997) reported magnetic levitation of frog embryos in a 13 T Bitter magnet with a gradient of ~100 T/m.

Prolonged magnetic levitation using resistive magnets is expensive and difficult to maintain. The Nijmegen Bitter magnet used 6 MW power supply that delivered 20 kA at 300 V. The magnet was cooled with 10°C H₂O flowing at 300 m³/h with an inlet pressure of 120 kPa. The electrical cost for running a similar Bitter magnet at the National High Magnetic Field Laboratory (NHMFL) in Tallahassee is \$14,000 (US\$) per day. These high operational costs preclude long-term levitation experiments that require many consecutive days or weeks of continuous operation.

There are a number of commercially produced room temperature bore superconducting magnets designed for long-term magnetic levitation of diamagnetic samples. Magnets made from superconductive wire can achieve high current density resulting in a strong magnetic field. The wire must be at a temperature below the wire's respective critical current and field density to maintain its superconductive properties, i.e., zero resistance. A reservoir of liquid helium bathes superconductive wire, which maintains the wire temperature at 4.2°K, which is below the temperature necessary for niobium tin or niobium titanium wire to be superconductive. Superconductive magnets are cost effective because once they are energized they can operate without a power supply or cooling supply. However, cryogens must be periodically refilled because the cryostat is not a perfect insulator and cryogens boiloff. Superconductive magnets designed for magnetic levitation having a warm bore less than 40 mm in diameter can operate in persistent mode at 4.2 K helium temperature. However, superconducting magnets with larger warm bores, e.g. greater than 50 mm, need to operate at a higher magnetic field because the inherent field gradient product is too low to support levitation. To obtain the necessary field-gradient product a lambda fridge is required to cool the liquid helium bath from 4.2 to 2.2 K so the magnet can operate at a higher magnetic field. Without additional helium cooling the University of Minnesota magnet would quench at 16 T, which is below the field-gradient product necessary for levitation of biological samples. The University of Minnesota lambda fridge consumes approximately 15 to 20 l of liquid helium per day to operate.

Maintenance of Mammalian Cells in a Levitation Magnet

The MC3T3-E1 osteoblastic cell line has a demonstrated sensitivity to gravitational loading conditions (Fitzgerald and Hughes-Fulford 1999). These cells were cultured in a 17 T/50 mm warm bore superconductive magnet (Oxford Instruments, Oxford, UK) to study the effect of a net gravitation force varying from 0 g through 2 g on gene expression. A bioreactor, similar to a liquid lift or fluidized bed reactor was designed to support cells in the levitation magnet for long-term studies. This configuration utilized MC3T3 cells grown on Cytodex-3 beads that were suspended in MEM-10% FBS media continually infused through a 3 ml container. The media bottle had 5% CO₂/95% air bubbled into the liquid for the experiment duration. The rate of media infusion was controlled by an eight-channel PumpPro MPL (Watson-Marlow Inc., Wilmington, MA, USA) peristaltic pump. Each vial had a dedicated channels to feed and evacuate the media. The feed rate was 200 µl/min and media removal rate was greater than 200 µl/min. The media inlet port was positioned lower than the outlet port to ensure the sample containers did not overflow. Employing an inlet flow rate of 200 µl/min is equivalent to adding four drops of media per minute, resulting in one complete volume exchange every 15 min. The cell perfusion method was designed to have minimal fluid shear, so as not to confound observed gene expression changes from magnetic levitation versus fluid shear effects. This perfusion method percolates media through the bead bed and exposes the cells to much less fluid shear than rotating bioreactors used for microgravity simulations.

The precise levitation zone was determined by placing the chamber where a water drop levitates. This was monitored with an Elmo MN42H (Elmo USA, Plainview, NY, USA) video camera located in the magnet bore. The magnet bore was maintained at $37 \pm 0.1^\circ\text{C}$ using a heat exchanger comprising of two concentric tubes with water passing through the space between the tubes controlled by a RTE-740D water reirculator (Thermo Scientific Waltham, MA, USA). Note that when 0 g and 2 g is specified in the data assume a deviation of ± 0.1 g from the stated value.

This experiment effectively simulates orbital freefall conditions because the magnetic force exactly balances the gravitational force. However, it should be noted that not all cellular components may experience the same force. This is because magnetic force is proportional to differences in magnetic susceptibility. Even though all of the cell will not experience exactly the same forces, it has been demonstrated that body forces for egg embryo components experience a force reduction of ten-fold from their non-levitated values (Valles et al. 1997).

Microarray Methodology

A Microarray screens thousands of genes for evidence of up regulation or down regulation of expression. Microarrays come in two versions. An array of thousands of oligonucleotide chains, 20 to 25 nucleotides in length, or cDNA fragments bound to a glass or nylon substrate. mRNA extracted from a tissue or cell line, is amplified, labeled with a fluorophore, and then hybridized to a microarray. The microarray is then scanned for evidence of hybridization via the intensity of the fluorophore as a function of position on the array. The array yields information about the quantity of mRNA in the cell. Genes that code for specific proteins are transcribed into specific messenger RNAs (mRNAs). Ribosomes assemble proteins based on message translated from mRNA. It is generally assumed that activated genes sequences result in mRNA transcription, whereas inactive/nonexpressed genes do not (though, there are exceptions to this rule). The microarray indirectly measures the presence of cellular mRNA, which ultimately codes for synthesized proteins. This allows us to measure the expression of both known genes and unknown genes or Sets (expressed

sequence tags). The relative intensity of a spot for two different cell populations is a measure of their respective gene expressions.

Microarrays clearly demonstrated differential gene expression for cortical renal cells cultured in a rotating bioreactor and in a non-rotating culture bag aboard the space shuttle, STS-90 "Neurolab" 15 day orbital flight (Hammond et al. 1999). More than 1,632 genes changed at steady state. The largest changes were found for genes coding for previously unknown stress response and heat shock proteins. Large changes were also noted for the vitamin D receptor and transcription factors.

Methods and Results

MC3T3-E1 osteoblastic cells (Sudo et al. 1983) were generously contributed by Rajaram Gopalakrishnan, BDS, Ph.D. (University of Minnesota; Minneapolis, MN, USA). Passage-20 cells were cultured to confluence in Earle's-modified MEM supplemented with Penicillin-Streptomycin, L-glutamine, and 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA). Cells were then removed from the tissue culture flasks by trypsinization, counted by hemocytometer, and added to 1 gm of hydrated Cytodex-3 microcarrier beads (Amersham Pharmacia Biotech; Piscataway, NJ, USA) in culture media. The bead/cell suspension was stirred and allowed to settle several times over the course of two hours in order to facilitate cell attachment to bead surfaces. Bead/cell suspensions were then stirred at a low rpm by a magnetic stir-plate in a tissue culture incubator for approximately 1 week. Cell proliferation was periodically assessed by examination of a small sample of beads under an inverted microscope.

When cell proliferation had progressed so that 5–10 cells were attached per bead, an aliquot of 5 ml of bead/cell suspension was transferred into three sterile polystyrene wells cut from a 24-well tissue culture plate. Culture media was used to top-off each vial, yielding a bead volume that was approximately 30% of the media volume contained in the well. The wells were then inserted into a vertically-configured jig designed to insert into the magnet's bore (Fig. 2).

Following 48 hours of culture within the magnet, wells were removed and beads immediately placed in Tri-Reagent RNA isolation reagent (MRC, Inc, Cincinnati, OH, USA). This solution separates total cellular RNA from protein and DNA by phenol and guanidine thiocyanate (as described by Chomczynski and Sacchi 1987). Following isolation, total RNA was purified through a silica membrane column (RNeasy; Qiagen Corp., Valencia, CA, USA), and quantified by spectrophotometry. Five micrograms of total RNA collected from each experimental condition were amplified by a MessageAmp II kit (Ambion, Inc., Austin, TX, USA). Briefly, this kit reverse transcribes the RNA to a first strand cDNA primed with a T7 oligo primer. This is then incubated with DNA polymerase in order to synthesize double stranded DNA (dsDNA), which in turn serves as a template for transcription. Following purification and the removal of RNA fragments, biotinylated RNA was amplified via transcription of the dsDNA in the presence of biotinylated UTP and CTP. The amplified RNA was purified, quantified by spectrophotometer, and quality checked on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

Amplified RNA (aRNA) was fragmented by a tris/magnesium/potassium acetates solution and 20 µg aRNA was loaded onto an Affymetrix Mouse Genome 430A 2.0 array chip (Santa Clara, CA, USA). This array chip contains 22,600 25-mer probe sets representing over 14,500 well-substantiated mouse genes. Hybridization and image scanning were carried out at the microarray core facility. The defective area, corner noise, outlier area, signal intensity, and 3':5' ratio of the β -actin and GAPD (glyceraldehyde-3-phosphate dehydrogenase)

housekeeping genes on the array were assessed for quality ensuring by Expressionist Pro4.5 (GeneData AG, CH-4016 Basel, Switzerland) Refiner module. The housekeeping genes are usually consistently expressed in different conditions to keep cells alive. A 3':5' ratio close to one indicates the mRNA is near intact since mRNA degrades from its 5' end. The probe level signals of each chip were condensed into probeset expression values by RMA algorithm (Irizarry et al. 2003).

The summarized data were scaled up to median of 100. The genes whose values are less than 50 in all samples were filtered out. Genes that changed more than three fold were tabulated in the sample comparisons and examined with respect to molecular function groups of these differentially expressed genes.

Based on Fig. 3 it appears that cells exposed to a net gravitational force of 0 or 2 g show a higher level of gene expression relative to cells exposed to a gravitational force of 1 g. A net gravitational force other than 1 g causes the cells to respond to this stress by exhibiting a higher level of gene expression. This has been seen for cells cultured in earth orbit (Carmelet et al. 1997,1998;Harris et al. 2000) or those exposed to hyper gravity (Hatton et al. 2003). Cells grown at the 0 g and 2 g magnet locations experience identical magnetic forces whereas cells grown in the magnet center do not experience a magnetic force because the spatial field gradient is negligible. It is also notable that samples at 0 g and 2 g magnet locations have a similar differential gene expression profile. One explanation is that since these cells are experiencing identical magnetic forces and that these forces are stressing the cells more than gravitational forces do. It is possible that intracellular forces occur due to intracellular organelles having different magnetic susceptibilities and thereby different net magnetic forces. This may manifest itself physiologically as internal stress or strain within the cell causing similar gene expression for cells experiencing 0 g and 2 g net forces. It is therefore possible there are magnetic effects that mask more subtle gravitational loading effects. To better understand what is occurring, it is necessary to study the expression level of specific biochemical markers.

Previous investigations of hyper and hypo gravity effects on cells or organisms have a demonstrable outcome. The fact that both lower and higher net gravitational fields affect gene expression is supported by comparing 2 g to 0 g gravity conditions. A small number of genes (195 genes), are different in this comparison leading one to conclude that these cells may be experiencing similar stresses.

While there are a relatively small number of genes differentially regulated when comparing the 0 g and 2 g expression data, it is critical to identify and evaluate the specific genes expressed. We found that the regulation of a number of genes are similarly impacted by both 0 g and 1 g including 1,425 up-regulated genes and 101 down-regulated. Some differences were apparent when comparing cells grown in 0 g and 2 g; 845 genes were specifically up-regulated in 0 g and 34 genes down-regulated, while 102 genes were up-regulated and 365 down-regulated in 2 g.

Table 1 shows that cells grown at 0 g (12.5 T) exhibit 2,270 genes up-regulated relative to cells at 1 g (17 T) whereas cells grown at 2 g (12.5 T) respond by up-regulating 1,527 genes relative to cells at 1g (17 T). The Venn diagram in Fig. 4 shows that 1,425 of the same genes from 0 g and 2 g were up-regulated. Fewer genes were down-regulated when comparing 0 g to 1 g, 135 genes, and 2 g to 1 g, 466 genes, where 101 genes were common to both 0 g and 2 g. However, this information does not define the functionality of the genes affected by gravitational stressors.

For functional gene analysis, gene probe set ID numbers were imported into the Ingenuity Pathway Analysis software (Ingenuity Systems, Inc., Redwood city, CA, USA). The

identified genes were related to functional groups by Fishers' *T* test. The more significantly affected genes are identified in Tables 2 and 3. The P values stand for how likely these genes belong to a function group by random chance alone. The smaller the P value, the more significant the identification of a particular gene. The most significant cell response to either hypo- or hyper-gravity is an increase in genes related to cell proliferation and cell death. These are obviously conflicting cell responses whereas one may be attributed to hypo-gravity and the other to hyper-gravity or a combination of gravitational and magnetic stresses. More experiments are necessary to better interpret these results.

Conclusion

Microarray analysis of gene regulation provides insight into simulated hypo- and hyper-gravity by magnetic levitation. Based on the limited data presented in this manuscript it appears that intracellular stress or strain due to magnetic and gravitational forces result in gene up-regulation in simulated 0 g (12.5 T) and 2 g (12.5 T) environments when compared to 1 g (17 T). Future studies are necessary with more replicates and different magnetic field strengths to disentangle gravitation from magnetic effects. Future studies need to include quantification of specific molecular and synthesized biomarkers via RT-PCR, Western blot, ELISA and/or RNase protection assays. Monitoring osteoblastic function under conditions where the net gravitational force is a variable, represents a unique venue for understanding the effect of gravitational forces on skeletal physiology. This is a new area of scientific inquiry that has not been explored because of limited access to magnet systems capable of magnetic levitation. Furthermore, with this instrument it is now possible to study biological function at 0.38 and 0.167 g, thus providing ground-based simulations of the gravitational forces experienced on the surface of Mars and the Moon, respectively.

Acknowledgments

The authors gratefully acknowledge support of this research through NIH grants 1R21EB003947 and 1S10RR16783-01, and the computational resources from Supercomputing Institute for Advanced Computational Research of the University of Minnesota.

Abbreviations

RT-PCR	reverse transcriptase-polymerase chain reaction
ELISA	enzyme-linked immunosorbent assay
RMA	robust multichip analysis
RNA	ribonucleic acid
T	Tesla

References

- Beaugnon E, Tournier R. Levitation of water and organic substances in high static magnetic fields. *J Phys III* 1991;1:1423–1428.
- Berry MV, Geim AK. Of flying frogs and levitrons. *Eur J Phys* 1997;18:307–313.
- Brooks JS, Reavis JA, Medwood RA, Stalcup TF, Meisel MW. New opportunities in science, materials, and biological systems in the low-gravity (magnetic levitation) environment. *J Appl Phys* 2000;87:6194–6199.
- Carneleit G, Nys G, Bouillon R. Microgravity reduces the differentiation of human osteoblastic MG-63 cells. *J Bone Miner Res* 1997;12(5):786–794. [PubMed: 9144345]
- Carmeliet G, Nys G, Stockmans I, Bouillon R. Gene expression related to the differentiation of osteoblastic cells is altered by microgravity. *Bone* 1998;22:139S–143S. [PubMed: 9600771]

- Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;162:156–159. [PubMed: 2440339]
- Coleman CG, Gonzalez-Villalobos RA, Allen PA, Johanson K, Guevorkian K, Valles JM, Hammond TG. Diamagnetic levitation changes, growth, cell cycle, and gene expression of *saccharomyces cerevisiae*. *Biotech Bioeng* 2007;98(4):854–863.
- Fitzgerald J, Hughes-Fulford M. Mechanically induced c-fos expression is mediated by cAMP in MC3T3-E1 osteoblasts. *FASEB J* 1999;13:553–557. [PubMed: 10064622]
- Guevorkian K, Valles JM. Swimming paramecium in magnetically simulated enhanced, reduced, and inverted gravity environments. *Appl Phys Lett* 2004;84(24):4863–4865.
- Hammond TG, Lewis FC, Goodwin TJ, Linnehan RM, Wolf DA, Hire KP, Campbell WC, Benes E, O'Reilly KC, Globus RK, Kaysen JH. Gene expression in space. *Nat Med* 1999;5:359–360. [PubMed: 10202906]
- Harris SA, Zhang M, Kidder LS, Evans GL, Spelsberg TC, Turner RT. Effects of orbital spaceflight on human osteoblastic cell physiology and gene expression. *Bone* 2000;26:325–331. [PubMed: 10719274]
- Hatton JP, Poodran M, Li CF, Luzzio C, Hughes-Fulford M. A short pulse of mechanical force induces gene expression and growth in MC3T3-E1 osteoblasts via an ERK 1/2 pathway. *J Bone Miner Res* 2003;18(1):58–66. [PubMed: 12510806]
- Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP. Summaries of affymetrix genechip probe level data. *Nucleic Acids Res* 2003;31:e15. [PubMed: 12582260]
- Jacobs CR, Yellowley CE, Davis BR, Zhou Z, Cimbala JM, Donahue HJ. Differential effects of steady versus oscillating flow on bone cells. *J Biomech* 1998;31:969–976. [PubMed: 9880053]
- Kaysen JH, Campbell WC, Majewski RR, Goda FO, Navar GL, Lewis FC, Goodwin TJ, Hammond TG. Select de novo gene and protein expression during renal epithelial cell culture in rotating wall vessels is shear stress dependent. *J Membr Biol* 1999;168:77–89. [PubMed: 10051691]
- Pavalko FM, Chen NX, Turner CH, Burr DB, Atkinson S, Hsieh YF, Qiu J, Duncan RL. Fluid shear-induced mechanical signaling in MC3T3-E1 osteoblasts requires cytoskeleton-integrin interactions. *Am J Physiol* 1998;275:C1591–C1601. [PubMed: 9843721]
- Sarkar D, Nagaya T, Koga K, Nomura Y, Gruener R, Seo H. Culture in vector-averaged gravity under clinostat rotation results in apoptosis of osteoblastic ROS 17/2.8 cells. *J Bone Miner Res* 2000;15(3):489–498. [PubMed: 10750563]
- Sudo H, Kodama HA, Amagi Y, Yamamoto S, Kasai S. In vitro differentiation and calcification in a new clonal osteogenic cell line derived from newborn mouse calvaria. *J Cell Biol* 1983;96(1):191–198. [PubMed: 6826647]
- Ueno S, Iwasaka M. Properties of diamagnetic fluid in high gradient magnetic fields. *J Appl Phys* 1994;75(10):7177–7179.
- Valles JM, Lin K, Denegre JM, Mowry KL. Stable magnetic field gradient levitation of *xenopus laevis*: toward low-gravity simulation. *Biophys J* 1997;73:1130–1133. [PubMed: 9251829]
- Valles JM, Wasserman SRRM, Schweidenback C, Edwardson J, Denegre JM, Mowry KL. Processes that occur before second cleavage determine third cleavage orientation in *xenopus*. *Exp Cell Res* 2002;274(1):112–118. [PubMed: 11855862]
- Yuge L, Hide I, Kumagai T, Kumei Y, Takeda S, Kanno M, Sugiyama M, Kataoka K. Cell differentiation and p38^{MARK} cascade are inhibited in human osteoblasts cultured in a three-dimensional clinostat. *In Vitro Cell Dev Biol-Animal* 2003;39:89–97.

Fig. 1.

Detailed view of magnet. Samples placed in the warm bore can experience a *magnetic force* from -1 to $+1$ g, depending on location. The magnetic force vector ($\mathbf{F}_m$) is collinear to the gravitational force vector ($\mathbf{F}_g$), i.e., vertical. The net gravitational force ($\mathbf{F}_{net}$) a specimen experiences ranges from 0 to 2 g, depending on location in the bore:

$$\begin{aligned} \text{At } z = +82.6 \text{ mm } \mathbf{F}_{net} &= \mathbf{F}_m + \mathbf{F}_g = -1 + 1 \text{ g} = 0 \text{ g} \\ \text{At } z = 0.0 \text{ mm } \mathbf{F}_{net} &= \mathbf{F}_m + \mathbf{F}_g = 0 \text{ g} + 1 \text{ g} = +1 \text{ g} \\ \text{At } z = -82.6 \text{ mm } \mathbf{F}_{net} &= \mathbf{F}_m + \mathbf{F}_g = +1 \text{ g} + 1 \text{ g} = +2 \text{ g} \end{aligned}$$

All z distance measurements are relative to the magnetic center

Fig. 2.
(A) Scanning electron micrograph of 200 μm diameter Cytodex-3 bead with varying degrees of MC3T3 cell coverage.
(B) Holder for positioning containers containing MC3T3 loaded Cytodex-3 beads.
(C) Close-up of cell container

Fig. 3.

Pairwise comparison of gene expressions in different gravity conditions. Both X-axis and Y-axis represent gene expression levels. The diagonal symmetric distribution of the gene expression values indicates most genes were not differentially expressed. Normal (symmetric) distribution, about the diagonal, also reflects good quality of data. **(A)** Gene expressions in MC3T3 cells at 0 g location ($B = 12.5$ T) were compared with those of the MC3T3 cells at the 1 g location ($B = 17$ T). **(B)** Gene expressions in MC3T3 cells at 2 g location ($B = 12.5$ T) were compared with those of the MC3T3 cells at the 1 g location ($B = 17$ T). **(C)** Lesser differentially expressed genes were found between in MC3T3 cells at 0 g location ($B = 12.5$ T) and in the MC3T3 cells at the 2 g location ($B = 12.5$ T)

Fig. 4. Venn diagrams showing up regulating and down regulating of genes under different net gravitational loads

Table 1

Genes that show a greater than threefold change in up regulation or down regulation

Sample comparison (g)	Differential genes number	Up regulated	Down regulated
0 to 1	2,405	2,270	135
2 to 1	1,993	1,527	466
2 to 0	195	10	185

Table 2

Most significant functional groups of up-regulated genes from cells experiencing 0 g or 2 g net force compared to 1 g

Groups	<i>P</i> value	# molecules
1,425 up-regulated genes common to cells experiencing 0 g or 2 g net force		
Cellular growth and proliferation	1.36E-12	223
Protein synthesis	5.82E-12	101
Cell death	4.48E-11	183
Cell cycle	4.15E-07	104
Cellular movement	2.56E-06	22
Connective tissue development and function	5.52E-06	20
Cellular assembly and organization	6.70E-06	24
Cellular function and maintenance	6.70E-06	24
Post-translational modification	1.73E-05	104
RNA post-transcriptional modification	2.53E-05	25
Protein degradation	4.84E-05	23
Most significant of 845 specific up-regulated genes for 0 g compared to 1 g		
Molecular transport	3.74E-05	23
Protein trafficking	3.74E-05	23
Cell death	5.79E-05	135
Most significant of 102 specific up-regulated genes for 2 g compared to 1 g		
Cell morphology	3.89E-05	2
Tissue morphology	1.16E-04	2
DNA replication, recombination, and repair	1.52E-04	3

Table 3

Most significant functional groups of down-regulated genes from cells experiencing 0 g or 2 g net force compared to 1 g

Category	Significance	# molecules
Most significant of 101 down-regulated genes common to cells experiencing 0 g or 2 g net force		
Cardiovascular system development and function	6.98E-05	2
Cellular development	6.98E-05	2
Hematological system development and function	6.98E-05	2
Gene expression	3.46E-04	2
Molecular transport	4.92E-04	3
Most significant of 34 down-regulated genes for 0 g compared to 1 g		
Viral function	8.27E-04	2
Cellular development	1.00E-03	4
Cell-to-cell signaling and interaction	1.88E-03	1
Most significant of 365 down-regulated genes for 2 g compared to 1 g		
Small molecule biochemistry	9.88E-03	5
Protein trafficking	4.61E-04	7