

Cells in Fluidic Environments Are Sensitive to Flow Frequency

MERCEDES BALCELLS,^{1,2*} MARTA FERNÁNDEZ SUÁREZ,^{1,2}
MARÍA VÁZQUEZ,^{1,2} AND ELAZER R. EDELMAN^{1,3}

¹Harvard-MIT Division of Health Sciences and Technology, Cambridge, Massachusetts

²Institut Químic de Sarrià, Ramon Llull University, Barcelona, Spain

³Cardiovascular Division, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts

Virtually all cells accommodate to their mechanical environment. In particular, cells subject to flow respond to rapid changes in fluid shear stress (SS), cyclic stretch (CS), and pressure. Recent studies have focused on the effect of pulsatility on cellular behavior. Since cells of many different tissue beds are constantly exposed to fluid flows over a narrow range of frequencies, we hypothesized that an intrinsic flow frequency that is optimal for determining cell phenotype exists. We report here that cells from various tissue beds (bovine aortic endothelial cells (BAEC), rat small intestine epithelial cells (RSIEC), and rat lung epithelial cells (RLEC)) proliferate maximally when cultured in a perfusion bioreactor under pulsatile conditions at a specific frequency, independent of the applied SS. Vascular endothelial and pulmonary epithelial cell proliferation peaked under 1 Hz pulsatile flow. In contrast, proliferation of gastrointestinal cells, which in their physiological context are subject to no flow or higher wavelength signal, was maximum at 0.125 Hz or under no flow. Moreover, exposure of BAEC to pulsatile flow of varying frequency influenced their nitric oxide synthase activity and prostacyclin production, which reached maximum values at 1 Hz. Notably, the “optimal” frequencies for the cell types examined correspond to the physiologic operating range of the organs from where they were initially derived. These findings suggest that frequency, independent of shear, is an essential determinant of cell response in pulsatile environments. *J. Cell. Physiol.* 204: 329–335, 2005. © 2005 Wiley-Liss, Inc.

Cells in tissue beds experience and respond to a mechanical environment that is defined by complex interactions between gravitational forces, local forces generated by the extracellular surroundings, and intracellular tension arising from the cytoskeletal organization (Helmke and Davies, 2002). Extensive work in animal and cell culture systems has identified a rapidly expanding list of mechanotransduced cellular responses, that translate mechanical stimuli into intracellular biochemical processes (Wirtz and Dobbs, 2000; Barbee, 2002; Bakker et al., 2003; Basson, 2003). Different cell types share a common sensitivity to mechanical stimuli that determines differentiation (Lamoureux et al., 2002; Lucchinetti et al., 2004; Mauney et al., 2004), rate of division (Vlahakis and Hubmayr, 2003), migration (Shreiber et al., 2003), gene expression (Davies et al., 2001; García-Cardena et al., 2001), secretion (Frangos et al., 1985; Baskin et al., 1993; Tilles et al., 2001), endocytosis (Truschel et al., 2002), and apoptosis (Graf et al., 2003), and use common molecular machinery for the regulation of these processes (Putnam et al., 1998).

Fluids flowing along tubular structures, and within interstitial spaces, create diverse patterns of shear stress (SS), which uniquely affect specific tissues and have clearly been identified as powerful cell control mechanism. Flow effects are determined by the magnitude, nature—steady, oscillatory, or pulsatile (Helmlinger et al., 1996; Li et al., 1998)—and spatial/temporal distribution of the flow. Exposure to tangential frictional forces activates flow-sensitive ion channels that hyperpolarize or depolarize the cell membrane in turn regulating a variety of cell responses (Blackman et al., 2000; García-Cardena et al., 2001). Such forces direct reorganization of cytoskeletal structure (Tzima et al., 2002; Essig and Friedlander, 2003), altered endocytosis (Apodaca, 2002), and production of prostaglandins

(Smalt et al., 1997; Norvell et al., 2004), growth factors (Waters et al., 1997; Passerini et al., 2003), and nitric oxide (Uematsu et al., 1995; McAllister et al., 2000; Bakker et al., 2001). In addition to the impact of SS, cells exposed to mechanical loads respond differentially to strain rates (Quinn et al., 2002; Drewes et al., 2003; Clark et al., 2004), number of loading cycles and periods of exposure (Robling et al., 2002; Lavagnino et al., 2003), and strain distribution and gradient (Judex et al., 1997; Helmke et al., 2003). Moreover, these parameters may act synergistically (Zhao et al., 1995) or in opposition to each other (Wang et al., 2001).

Many organs are subject to pulsatile forces, at frequencies that are organ-, context-, and species-specific. Respiration rate, for example, scales with size such that the normal breathing rate in the rat is five times faster than the human pace (12–17 breaths per min) (Huang et al., 2000). Analogously, the adult human resting heart rate is half that of the human embryo (Makrydimas et al., 1997; Oncken et al., 2002). Tissue generation, function, injury, repair, and regeneration may be differently modulated by all these frequencies. The dearth of references, however, that deal with the impact of flow on non-vascular cells (Anderson and Vora, 1995; Bhat et al., 1995; Ajubi et al., 1996; Van der Pauw

Contract grant sponsor: National Institutes of Health; Contract grant numbers: HL60407, HL67246, and GM/HL49039.

*Correspondence to: Mercedes Balcells, Harvard-MIT Division of Health Sciences and Technology, 77 Massachusetts Avenue, Building 56-322, Cambridge, MA 02139. E-mail: merche@mit.edu

Received 18 October 2004; Accepted 12 November 2004

DOI: 10.1002/jcp.20281

et al., 2000; Nauman et al., 2001; Torok et al., 2001; Tanaka et al., 2003; Warden and Turner, 2004) stands in stark contrast to the extensive literature on this aspect of vascular cell physiology (for reviews see Fisher et al., 2001; Gimbrone et al., 2002; Resnick et al., 2003).

The present study investigates the role of frequency on cellular proliferation and metabolic function. We hypothesized that cells that reside in organs subject to physiologic flow conditions respond to particular frequencies that optimally enhance cell phenotype and maintain cells functionally differentiated. Furthermore, we expected the inherent "optimal" frequencies fall within the narrow range of the physiological frequencies that affect the tissue beds in which the cells in question normally reside. By examining the frequency-dependent proliferation of cells from four different organs exposed to pulsatile stimuli similar to those encountered in their physiological context, we showed that cells respond to particular flow frequencies independent of average shear.

MATERIALS AND METHODS

Physical description of the perfusion system

The perfusion bioreactor (Fig. 1) is comprised of eight independent channels of tubing forming closed loops, each divided into three segments (inlet, test, and outlet segment). In every channel, perfusate (cell culture medium, described below) flowed from a custom-made glass reservoir through a 40 cm-inlet length silicone rubber tubing (L_{in} , Silastic[®] laboratory tubing, 4.78 mm ID \times 7.92 mm OD, Dow Corning, Midland, MI), and thereafter circulated through the 9 cm-length test segment containing the cultured cells. After passing over the test segment, the medium flowed through a 40 cm-outlet length tubing (L_{out}), and was returned to the reservoir via 20 cm of the same silicone rubber tubing. The entire apparatus was placed in an incubator at 37°C under 10%

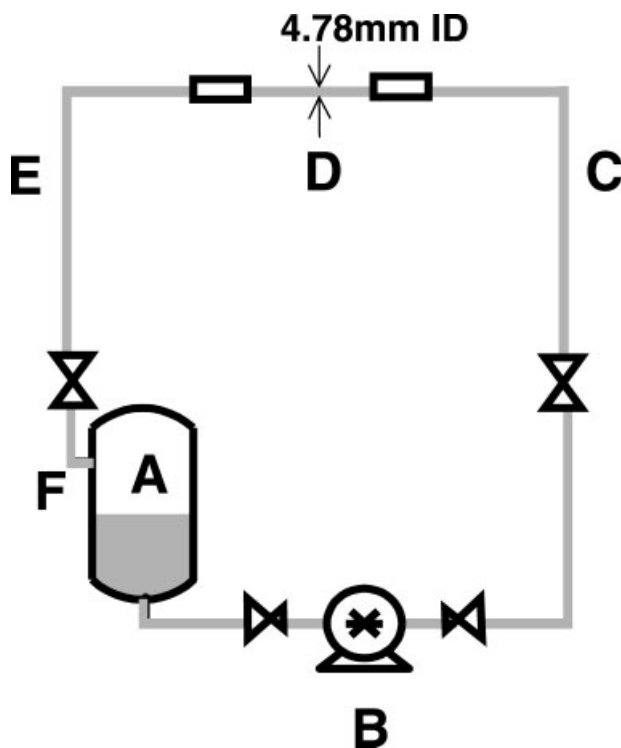


Fig. 1. Schematic diagram of the pulsatile perfusion system. A peristaltic pump (B) propelled perfusate from a reservoir (A) through the inlet length tubing (C), and test segments containing the cultured cells (D). Medium flowed through the outlet length tubing (E), and returned to the reservoir via 20 cm of the same silicone rubber tubing (F).

CO₂. The gas permeability of the Silastic tubing enabled the maintenance of pH and oxygen exchange into the perfused media, which were confirmed with direct measurements.

A programmable peristaltic Ismatec[®] pump (Cole-Parmer, Vernon Hills, IL) propelled the media from 1.5 to 177 ml/min at a maximum pressure of 22 psi. A custom-developed analog circuit designed to generate a 0–5 VDC square waveform, with a 60% duty cycle, allowed the regulation of the flow profile. An ultrasound flowmeter (Transonic Animal Research, Ithaca, NY) monitored instantaneous and average volumetric flow. Small changes in vessel diameter were recorded using a digital camera connected to a light epimicroscope. The pressure gradient within the test tube was measured using a custom-made differential pressure meter consisting of a differential pressure sensor (Motorola, MPX10DP, Andover, MA) and a 324 Quad operational-amplifier chip. These data were processed through an A/D converter, and stored for off-line analysis on a digital computer.

Cell culture

Bovine aortic endothelial cells (BAEC) (CSL certified, Kirkland, WA), rat lung epithelial cells (RLEC) (ATCC, Manassas, VA), and rat small intestine epithelial cells (RSIEC) (ATCC) were cultured in DMEM (Gibco, Grand Island, NY) supplemented with 10% (v/v) fetal bovine serum (FBS, HyClone, Logan, UT), 35 μ M streptomycin sulfate (S, Gibco), 50 U/ml penicillin (P, Gibco), and 2 mM glutamine (G, Gibco). Human umbilical vein endothelial cells (HUVEC) (Cambrex Bio Science, Walkersville, MD) were cultured in EBM-2 medium (Cambrex) supplemented with 7% (v/v) FBS and the bullet kit of growth factors and cytokines (CC-3162) as recommended by the manufacturer. Primary cultures were established, maintained, and passaged to passage 4 on tissue culture polystyrene (TCPS) plates. Test segments were prepared as follows: 14 cm-length Silastic[®] laboratory tubes (ID = 4.78 mm, OD = 7.92 mm, Dow Corning) were rinsed with successive 20 min incubations in 0.2% SDS solution (Fluka, Steinheim, Germany), and distilled water in an ultrasonic bath. After autoclave sterilization of the tubes, the Silastic[®] tubes were coated with 100 μ g/ml bovine fibronectin (Sigma-Aldrich, St. Louis, MO) in PBS for 2 h while rotating at 10 rpm at 37°C. Subsequently, BAEC, RLEC, RSIEC, or HUVEC were seeded onto the inner surface of the fibronectin pre-coated tubing at 2.3×10^4 cell/cm² (30% confluent) and rotated axially at 10 rpm while in incubation for 24 h.

The behavior of BAEC, RSIEC, and RLEC was examined within a frequency range of 0–1.5, 0–0.5, and 0–1.5 Hz, respectively. Those intervals were chosen based on an average bovine heart rate of approximately 1 Hz (Lefcourt et al., 1999), rat small intestine contraction rate of 0–0.4 Hz (Bercik et al., 1994; Krantis et al., 1996), and rat respiratory frequency of 1 Hz (Huang et al., 2000). As a control of cell type that is exposed to laminar steady flow under healthy physiological conditions (Hofstaetter et al., 2001), HUVEC proliferation was also studied within the interval 0–1.5 Hz.

Proliferation assays

Cell number was measured after trypsinization using a particle counter (Beckman Coulter Corporation, Miami, FL). The initial attached cell number was determined 24 h after seeding (day 0). The remaining seeded tubes were either placed within the perfusion system for additional 24–48 h under flow, or mounted in the system without flow as a sham control. FACS analysis using anti-Ki-67 antigen (Dako, Glostrup, Denmark) was performed to assess the percentage of cells in active phases of the cell cycle. The trypan blue exclusion assay assessed the viability of cells after passage and culture. Homogenous seeding and maintenance of cell adhesion was validated at various points in time through direct examination of the tubes and sampling of the perfusate.

Endothelial nitric oxide synthase (eNOS) activity and prostacyclin (PGI₂) production

A 6-keto-prostaglandin F_{1 α} enzyme-immunoassay (EIA) system (Amersham, Woburn, MA) was assayed for PGI₂ in serum-free perfusate collected after 24 h of cell exposure to

pulsatile flow. eNOS activity in cell lysates, obtained by EDTA (1 mM)/Tris (25 mM) treatment followed by sonication immediately after cessation of 24 h flow exposure, was determined using the [^3H]-L-arginine/L-citrulline assay kit (Cayman Chemical, Ann Arbor, MI). Results were normalized to the total amount of protein determined by the BCA protein assay kit (Pierce, Rockford, IL).

Statistical analysis

All experiments were carried out in quadruplicate, and data reported as the mean \pm SD. Statistical analysis was performed by ANOVA for repeated measures followed by a non-paired *t*-test. Values of $P < 0.05$ (two-tailed analysis) were considered significant.

RESULTS

System validation

Mean SS and cyclic stretch (CS) remained constant over the range of applied frequencies. Cells were exposed to pulsatile flows of two different mean SS values, 1.35 ± 0.1 and 5.11 ± 0.41 dyn/cm 2 , with average flow values of 86.7 ± 5.3 and 360.4 ± 18.3 ml/min, respectively. At low flow frequencies, the flow profile followed closely the input electric signal, a square wave of 60% duty cycle (Fig. 2A). As the frequency increased, the profile became more sinusoidal (Fig. 2C). A Womersley-type approximation, which considered the axial translation of the tube wall under pulsatile flow conditions (Moore et al., 1994b), was applied to estimate the velocity profiles (see Appendix). The velocity profiles obtained were parabolic for all frequencies independent of the flow profile (Fig. 2B, D) and allowed calculation of

the instantaneous wall SS for each time point of the cycle. Average of the instantaneous SS values in a cycle verified that mean SS did not change for all flow frequencies studied. The observed minor high frequency oscillations (Fig. 2A, C) originating from the eight rollers of the peristaltic pump head were present in all cases and were not considered a source of data variability. Additionally, no significant dimensional changes in the test segments were detected with flow exposure. Tube diameters were constant and CS invariant with only a $0.30\% \pm 0.05\%$ change across frequencies.

Cell proliferation and metabolic function

At a mean SS of 1.35 ± 0.10 dyn/cm 2 , each cell type studied presented a similar trend; proliferation was indistinguishable from the steady flow case at all flow frequencies except within a narrow frequency range (Fig. 3). Cells were able to discriminate as little as a 4% decrease of the maximum frequency value applied. BAEC and RLEC proliferated maximally at a frequency value of 1 Hz, while RSIEC did so at 0.125 Hz or under no flow conditions. These "optimal" frequencies fall within the range of the natural frequencies for flow through the correspondent tissue beds. Cell numbers, after exposure to the optimum frequencies, increased two- to three-fold for all cells 24 and 48 h after seeding and flow exposure. Interestingly, at the optimal frequency BAEC and RLEC became confluent after 24 h, indicating a faster doubling time than the 20 h period observed on TCPS static cultures. At other frequency values, cells

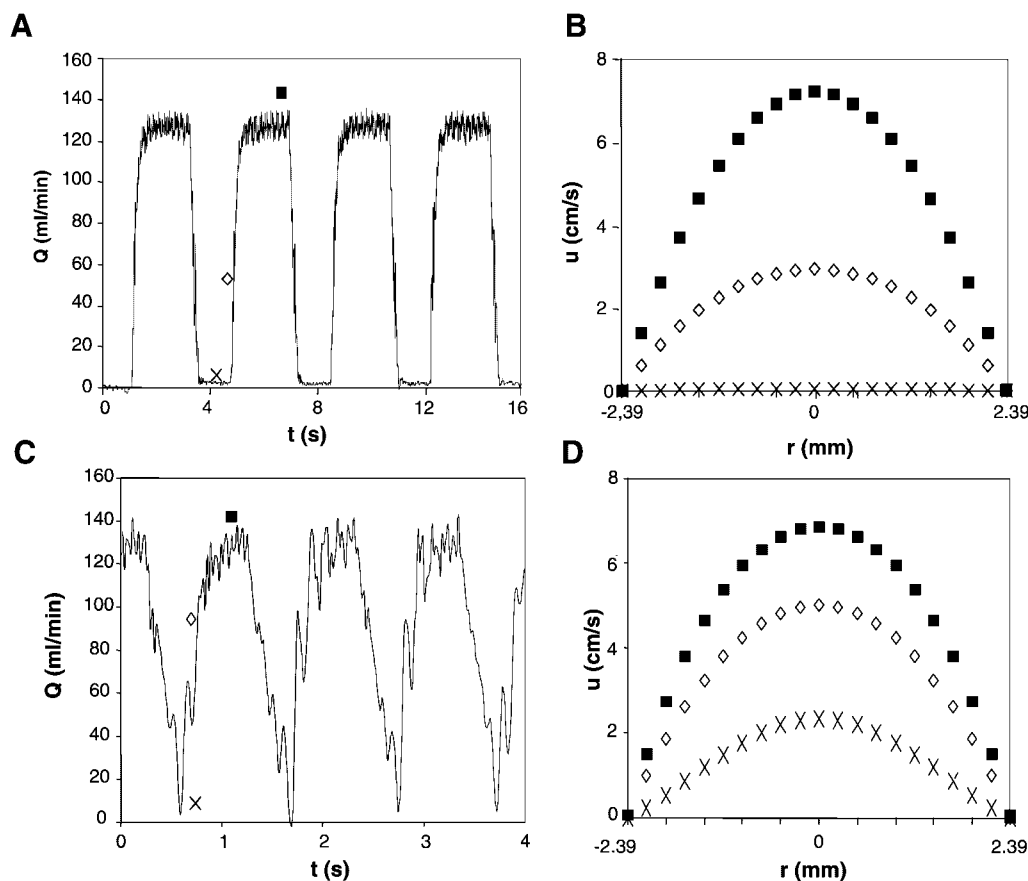


Fig. 2. Flow and velocity profiles obtained at 0.25 Hz (A, B) and 0.99 Hz (C, D). The profiles shown were obtained at average shear stress (SS) of 1.35 ± 0.1 dyn/cm 2 . Each velocity profile shown corresponds to a relevant time point in the flow cycle indicated by the symbols ■, ◇, X.

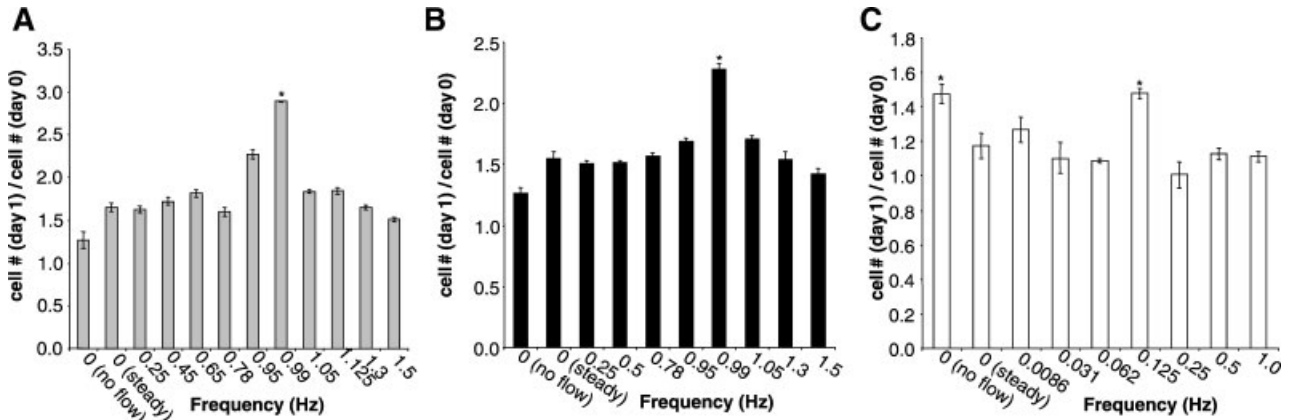


Fig. 3. Cell numbers after 24 h flow exposure. Ratio between cell numbers at day 1 and 0 measured on bovine aortic endothelial cells (BAEC) (A), rat lung epithelial cells (RLEC) (B), and rat small intestine epithelial cells (RSIEC) (C) subjected to no flow, steady flow, and pulsatile flow of varying frequencies. Cell number measured at

day 0 was $3.6 \pm 0.13 \times 10^5$. Flow experiments were carried out at average SS of $1.35 \pm 0.1 \text{ dyn/cm}^2$. $P < 0.05$ comparison made between value of proliferation quotient at a given frequency value and that obtained at f_{max} .

continued to proliferate, but at a slower rate and did not reach confluency within the duration of the experiments. Similarly, RSIEC did not reach confluency during the length of the experiments under any condition applied. The number of unattached cells in the

perfused culture medium 24 h after flow exposure remained less than 5% of the total attached cell number for all investigated frequencies, and was statistically indistinguishable from the number of unattached cells observed in controls under no flow. Accordingly, cell

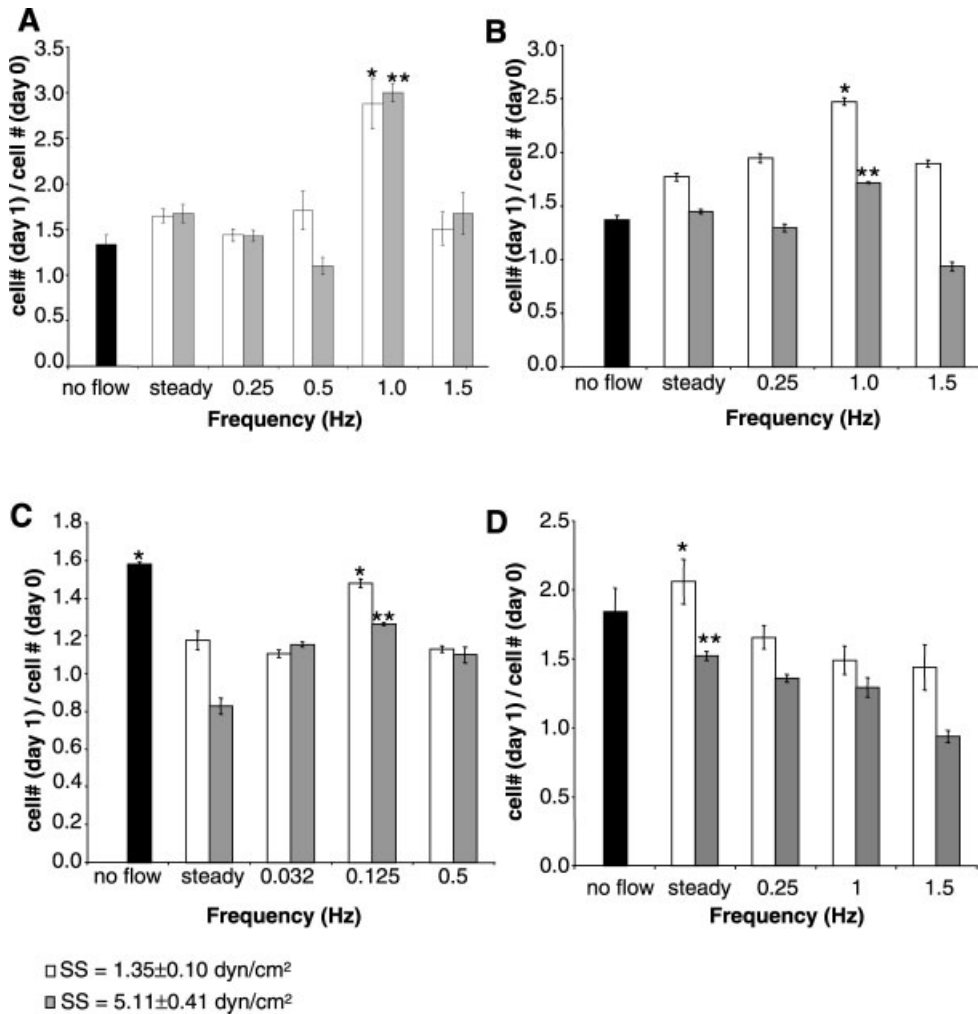


Fig. 4. Cell proliferation at low and high mean SS and different flow frequencies for BAEC (A), RLEC (B), RSIEC (C), and human umbilical vein endothelial cells (HUVEC) (D). $P < 0.05$ comparison made between value of proliferation quotient at a given frequency value and that obtained at f_{max} .

detachment was regarded as having an insignificant effect on observed changes in cell number.

In an additional set of experiments, cells were subjected to a higher mean SS of 5.11 ± 0.41 dyn/cm² (Fig. 4A–C). Similar to the lower mean SS case, maximum proliferation was achieved at 1, 1, and 1/8 Hz flow frequency for BAEC, RLEC, and RSIEC, respectively. At other frequency values, there was no statistically significant difference in proliferation compared to the steady flow case. Given that flow in the umbilical vein is non-pulsatile under normal physiological conditions (Hofstaetter et al., 2001), HUVEC served as an additional control. HUVEC proliferated maximally under steady flow conditions under the two levels of SS applied (Fig. 4D).

FACS analysis of Ki-67 positive cells, eNOS activity, and PGI₂ production of BAEC exposed at different frequencies at the low SS level previously applied mirrored the same trend as the cell counts, with maximum proliferation, enzyme conversion activity and PGI₂ secretion at 1 Hz (Fig. 5A–C).

DISCUSSION

It is well established that fluid pulsatility, together with SS and CS, modulates cellular behavior [Fisher et al., 2001; Gimbrone et al., 2002; Resnick et al., 2003 for reviews]. Our study now shows a flow frequency-dependent change in cell biology independent of mean SS and total flow. The calculated average SS, CS, and total flow were constant for all flow patterns applied across frequencies. All cells examined were constituent of a tissue or organ exposed under physiologic conditions to flows, and their optimal biologic responsiveness appears to center around the frequency of organ operation. Aortic endothelial and lung epithelial cells showed increased cell number within the narrow frequency range of blood flow through vessels and air flow through bronchi. Proliferation of intestinal epithelial cells in contrast was maximum under two distinctive conditions, no flow and 0.125 Hz. This result is entirely consistent with the dual nature of gastrointestinal physiology, which, unlike the vasculature or bronchi, possesses two operative states: a quiescent or rest state where no contraction is observed, and an active state with contractile frequency of approximately 0.125 Hz. Finally, HUVEC, which served as a non-pulsatile cell system, showed maximum proliferation under steady flow conditions reinforcing our hypothesis and validat-

ing our methods. eNOS activity and prostacyclin production by BAEC similarly track with frequency. These data constitute a preliminary indication that signaling and metabolic events, like proliferation, are optimized at the inherent frequencies to which they are exposed in their physiologic environments.

Frequency-dependent cell function has important implications for a variety of sciences including tissue generation, regeneration, and engineering. One might now envision that specific congenital anomalies could arise as a result of alterations in flow frequency. Similarly the remodeling of diseased tissue may follow not only stress and stretch but frequency as well. The concept of tachycardia-mediated cardiomyopathy is well appreciated. But of immediate interest as well is the role these findings might play in tissue engineering (Langer and Vacanti, 1993). The development of biological substitutes to restore, maintain, or improve tissue function currently employs cells within porous scaffolds. The in vitro culture of cell-scaffold constructs under conditions that support efficient nutrition of cells, combined with the application of mechanical forces to direct cellular activity and phenotype, is an important step towards the development of functional grafts for the treatment of lost or damaged body parts (Niklason et al., 1999; Butler et al., 2000; Raimondi et al., 2002). In this context, bioreactors provide controlled environments for reproducible and accurate application of specific regimes of mechanical forces to cell constructs (Moore et al., 1994a; Peng et al., 2000; Qiu and Tarbell, 2000; Blackman et al., 2002; Guyot and Hanrahan, 2002). Our study presents an alternative to high density/high efficiency cell seeding towards the generation of confluent functional cell implants. Exposure of sub-confluent cell constructs to optimum pulsatile conditions enhanced cell proliferation. Thus, conventional cell culturing conditions may be enhanced with the consideration of input frequency and SS, narrowing the gap between the physiological and in vitro conditions.

By studying flow frequency independent of other environmental stimuli, we have added a new parameter to the existing body of knowledge on the effect of mechanical forces regarding the behavior of sub-confluent cells. There appears to be an intrinsic frequency for cells residing in pulsatile flow conditions that affects functionality and viability. We hope to leverage our data to further elucidate the underlying mechanisms that govern mechanically induced cell signaling and

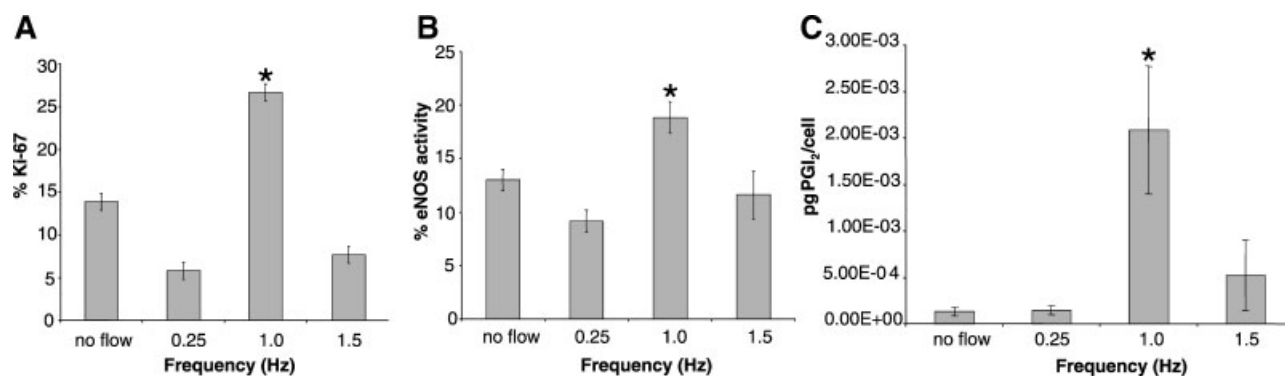


Fig. 5. Effect of flow frequency on BAEC proliferation (A), endothelial nitric oxide synthase (eNOS) activity (B), and prostacyclin (PGI₂) production (C). Data obtained after exposure to a frequency below the optimum (0.25 Hz), at the optimum (1 Hz), and at a frequency higher than the optimum (1.5 Hz). Shear stress applied was 1.35 ± 0.1 dyn/cm². The extend of proliferation is given as measure of Ki-67

positive cells by FACS analysis, eNOS activity is expressed as percentage of enzyme conversion normalized to total protein produced per cell lysate and PGI₂ production is measured in the conditioned medium and normalized per total cell number. $P < 0.05$ comparison made between value at a given frequency value and that obtained at f_{max} .

proliferation by investigating the role of frequency in the direct expression of other biochemical and transcriptional events.

ACKNOWLEDGMENTS

We thank Drs. Rami Tzafri, Shai Schubert, David Ettenson, Yoram Richter, Carlos Semino, Kumaran Kolandaivelu, and Mss. Blanca San Miguel, and Blanca Molins for their helpful advice. This work was supported in part by the National Institutes of Health (HL60407, HL67246, and GM/HL49039). Ms. M. Vázquez thanks Fundación Caixa-Galicia for their support through a year fellowship.

APPENDIX

Characterization of the perfusion system: Entrance length and SS evaluation

The Reynolds number (Re) represents the ratio of inertial to viscous forces. For a fluid flowing through a cylindrical tube:

$$Re = \rho \cdot D \cdot V / \mu \quad (1)$$

where D is the tube diameter, V is the fluid velocity, ρ is the density, and μ the dynamic viscosity. The segments L_{in} and L_{out} in our system correspond to the inlet and outlet lengths, respectively. The inlet length of a tube is the distance over which the axial velocity profile develops to within a small percentage of its ultimate shape. A conservative estimate for the unsteady inlet length is given by:

$$L = n \cdot r \cdot (Re) \quad (2)$$

where r is the radius, (Re) the Reynolds number, and n a numerical factor derived mathematically (Fung, 1984). Since the approach to parabolic profile is asymptotic, there is no unique value of n , thus many values can be found in the literature (Caro et al., 1978, McDonald, 1974). Applying Eq. 2 using the value of n recommended in the latter reference (McDonald, 1974) and the maximum Reynolds number of 2,100; 40 cm were obtained as optimum inlet and outlet length.

SS was calculated following a Womersley-type solution, assuming laminar flow of a Newtonian fluid through a straight rigid tube with a periodic axial pulsatile pressure gradient (85). Under these conditions, the solution for the instantaneous axial velocity profile is given by:

$$u(r, t) = \frac{-G_0}{4\nu} (R^2 - r^2) + \sum_{n=1}^{\infty} \frac{G_n}{in\omega} \left[1 - \frac{J_0(i^{3/2}\alpha_n r/R)}{J_0(i^{3/2}\alpha_n)} \right] e^{in\omega t} \quad (3)$$

where R is the radius of the tube, ω is the fundamental angular frequency, and ν is the kinematic viscosity.

The constants G_n are determined by the Fourier coefficients of the pressure gradient waveform:

$$\nabla P = \sum_{n=0}^{\infty} G_n e^{in\omega t} \quad (4)$$

and

$$\alpha_n = R \sqrt{n\omega/\nu} \quad (5)$$

This waveform can then be used to calculate the instantaneous SS according to:

$$\tau(t) = \mu \left. \frac{\partial u(r, t)}{\partial r} \right|_{r=R} \quad (6)$$

where μ is the dynamic viscosity.

LITERATURE CITED

- Ajubi NE, Klein-Nulend J, Nijweide PJ, Vrijheid-Lammers T, Alblas MJ, Burger EH. 1996. Pulsating fluid flow increases prostaglandin production by cultured chicken osteocytes—A cytoskeleton-dependent process. *Biochem Biophys Res Commun* 225(1):62–68.
- Anderson S, Vora JP. 1995. Current concepts of renal hemodynamics in diabetes. *J Diabetes Complications* 9(4):304–307.
- Apodaca G. 2002. Modulation of membrane traffic by mechanical stimuli. *Am J Physiol Renal Physiol* 282(2):F179–F190.
- Bakker AD, Soejima K, Klein-Nulend J, Burger EH. 2001. The production of nitric oxide and prostaglandin E(2) by primary bone cells is shear stress dependent. *J Biomech* 34:671–677.
- Bakker AD, Klein-Nulend J, Burger EH. 2003. Mechanotransduction in bone cells proceeds via activation of COX-2, but not COX-1. *Biochem Biophys Res Commun* 305(3):677–683.
- Barbee KA. 2002. Role of subcellular shear-stress distributions in endothelial cell mechanotransduction. *Ann Biomed Eng* 30(4):472–482.
- Baskin L, Howard PS, Macarak E. 1993. Effect of physical forces on bladder smooth muscle and urothelium. *J Urol* 150(2 Pt 2):601–607.
- Basson MD. 2003. Paradigms for mechanical signal transduction in the intestinal epithelium. *Digestion* 68(4):217–225.
- Bercik P, Armstrong D, Fraser R, Dutoit P, Emde C, Primi MP, Blum AL, Kucera P. 1994. Origins of motility patterns in isolated arterially perfused rat intestine. *Gastroenterology* 106:649–657.
- Bhat VD, Windridge PA, Cherry RS, Mandel LJ. 1995. Fluctuating shear stress effects on stress fiber architecture and energy metabolism of cultured renal cells. *Biotechnol Prog* 11(5):596–600.
- Blackman BR, Thibault LE, Barbee KA. 2000. Selective modulation of endothelial cell $[Ca^{2+}]_i$ response to flow by the onset rate of shear stress. *Biomech Eng* 122(3):274–282.
- Blackman BR, Garcia-Cardena G, Gimbrone MA, Jr. 2002. A new in vitro model to evaluate differential responses of endothelial cells to simulated arterial shear stress waveforms. *J Biomech Eng* 124(4):397–407.
- Butler DL, Goldstein SA, Guilak F. 2000. Functional tissue engineering: The role of biomechanics. *J Biomech Eng* 122:570–575.
- Caro CG, Pedley TJ, Schroter RC, Seed WA. 1978. In: *The mechanics of the circulation*. Oxford, New York: Oxford University Press. pp. 44–78.
- Clark CB, McKnight NL, Frangos JA. 2004. Stretch activation of GTP-binding proteins in C2C12 myoblasts. *Exp Cell Res* 292(2):265–273.
- Davies PF, Shi C, Depaola N, Helmke BP, Polacek DC. 2001. Hemodynamics and the focal origin of atherosclerosis: A spatial approach to endothelial structure, gene expression, and function. *Ann NY Acad Sci* 947:7–16.
- Drewes AM, Pedersen J, Liu W, Arendt-Nielsen L, Gregersen H. 2003. Controlled mechanical distension of the human oesophagus: Sensory and biomechanical findings. *Scand J Gastroenterol* 38(1):27–35.
- Essig M, Friedlander G. 2003. Tubular shear stress and phenotype of renal proximal tubular cells. *J Am Soc Nephrol* 14(Suppl 1):S33–S35.
- Fisher AB, Chien S, Barakat AI, Nerem RM. 2001. Endothelial cellular response to altered shear stress. *Am J Physiol Lung Cell Mol Physiol* 281(3):L529–L533.
- Frangos JA, Eskin SG, McIntire LV. 1985. Flow effects on prostacyclin production by cultured human endothelial cells. *Science* 243:1477–1479.
- Fung YC. 1984. In *biodynamics: Circulation*. New York: Springer. pp. 77–165.
- Garcia-Cardena G, Comander J, Anderson KR, Blackman BR, Gimbrone MA, Jr. 2001. Biomechanical activation of vascular endothelium as a determinant of its functional phenotype. *Proc Natl Acad Sci USA* 98:4478–4485.
- Gimbrone MA, Jr., Topper JN, Nagel T, Anderson KR, Garcia-Cardena G. 2002. Endothelial dysfunction, hemodynamic forces, and atherogenesis. *Ann NY Acad Sci* 902:230–239.
- Graf R, Apenberg S, Freyberg M, Friedl P. 2003. A common mechanism for the mechanosensitive regulation of apoptosis in different cell types and for different mechanical stimuli. *Apoptosis* 8(5):531–538.
- Guyot A, Hanrahan JW. 2002. ATP release from human airway epithelial cells studied using a capillary cell culture system. *J Physiol* 545(Pt 1):199–206.
- Helmke BP, Davies PF. 2002. The cytoskeleton under external fluid mechanical forces: Hemodynamic forces acting on the endothelium. *Ann Biomed Eng* 30(3):284–296.
- Helmke BP, Rosen AB, Davies PF. 2003. Mapping mechanical strain of an endogenous cytoskeletal network in living endothelial cells. *Biophys J* 84(4):2691–2699.
- Helmlinger G, Berk BC, Nerem RM. 1996. Pulsatile and steady flow-induced calcium oscillations in single cultured endothelial cells. *J Vasc Res* 33(5):360–369.
- Hofstaetter C, Dubiel M, Gudmundsson S. 2001. Two types of umbilical venous pulsations and outcome of high-risk pregnancy. *Early Hum Dev* 61(2):111–117.
- Huang ZG, Subramanian SH, Balnave RJ, Turman AB, Moi Chow C. 2000. Roles of periaqueductal gray and nucleus tractus solitarius in cardiorespiratory function in the rat brainstem. *Respir Physiol* 120(3):185–195.
- Judex S, Gross TS, Zernicke RF. 1997. Strain gradients correlate with sites of exercise-induced bone-forming surfaces in the adult skeleton. *J Bone Miner Res* 12:1737–1745.
- Krantz A, Glasgow I, McKay AE, Mattar K, Johnson F. 1996. A method for simultaneous recording and assessment of gut contractions and relaxations in vivo. *Can J Physiol Pharmacol* 74(8):894–903.
- Lamoureux P, Ruthel G, Buxbaum RE, Heidemann SR. 2002. Mechanical tension can specify axonal fate in hippocampal neurons. *J Cell Biol* 159(3):499–508.
- Langer R, Vacanti JP. 1993. Tissue engineering. *Science* 260(5110):920–926.

- Lavagnino M, Arnoczky SP, Tian T, Vaupel Z. 2003. Effect of amplitude and frequency of cyclic tensile strain on the inhibition of MMP-1 mRNA expression in tendon cells: An in vitro study. *Connect Tissue Res* 44(3-4):181-187.
- Lefcourt AM, Erez B, Varner MA, Barfield R, Tasch U. 1999. A noninvasive radiotelemetry system to monitor heart rate for assessing stress responses of bovines. *J Dairy Sci* 82(6):1179-1187.
- Li Q, Muragaki Y, Hatamura I, Ueno H, Ooshima A. 1998. Stretch-induced collagen synthesis in cultured smooth muscle cells from rabbit aortic media and a possible involvement of angiotensin II and transforming growth factor-beta. *J Vasc Res* 35(2):93-103.
- Lucchinetti E, Bhargava MM, Torzilli PA. 2004. The effect of mechanical load on integrin subunits alpha5 and beta1 in chondrocytes from mature and immature cartilage explants. *Cell Tissue Res* 315(3):385-391.
- Makrydimas G, Lolis D, Georgiou I, Skendou C, Nicolaidis KH. 1997. Fetal heart rate following coelocentesis. *J Matern Fetal Med* 6(6):314-316.
- Mauney JR, Sjostrom S, Blumberg J, Horan R, O'Leary JP, Vunjak-Novakovic G, Volloch V, Kaplan DL. 2004. Mechanical stimulation promotes osteogenic differentiation of human bone marrow stromal cells on 3-D partially demineralised bone scaffolds in vitro. *Calcif Tissue Int* 74(5):458-468.
- McAllister TN, Du T, Frangos JA. 2000. Fluid shear stress stimulates prostaglandin and nitric oxide release in bone marrow-derived preosteoclast-like cells. *Biochem Biophys Res Commun* 270(2):643-648.
- McDonald DA. 1974. In blood flood in arteries. Baltimore: Williams & Wilkins. pp. 101-117.
- Moore JE, Jr., Burki E, Suci A, Zhao S, Burnier M, Brunner HR, Meister JJ. 1994a. A device for subjecting vascular endothelial cells to both fluid shear stress and circumferential cyclic stretch. *Ann Biomed Eng* 22:416-422.
- Moore JE, Jr., Guggenheim N, Delino A, Doriot PA, Dorsaz PA, Rutishauser W, Meister JJ. 1994b. Preliminary analysis of the effects of blood vessel movement on blood flow patterns in the coronary arteries. *J Biomech Eng* 116:302-306.
- Nauman EA, Satcher RL, Keaveny TM, Halloran BP, Bikle DD. 2001. Osteoblasts respond to pulsatile fluid flow with short-term increases in PGE2 but no change in mineralisation. *J Appl Physiol* 90(5):1849-1854.
- Niklason LE, Gao J, Abbott WM, Hirschi KK, Houser S, Marini R, Langer R. 1999. Functional arteries grown in vitro. *Science* 284:489-493.
- Norvell SM, Ponik SM, Bowen DK, Gerard R, Pavalko FM. 2004. Fluid shear stress induction of COX-2 protein and prostaglandin release in cultured MC3T3-E1 osteoblasts does not require intact microfilaments or microtubules. *J Appl Physiol* 96(3):957-966.
- Oncken C, Kranzler H, O'Malley P, Gendreau P, Campbell WA. 2002. The effect of cigarette smoking on fetal heart rate characteristics. *Obstet Gynecol* 99(5 Pt 1):751-755.
- Passerini AG, Milsted A, Rittgers SE. 2003. Shear stress magnitude and directionality modulate growth factor gene expression in preconditioned vascular endothelial cells. *J Vasc Surg* 37(1):182-190.
- Peng X, Recchia FA, Byrne BJ, Wittstein IS, Ziegelstein RC, Kass DA. 2000. In vitro system to study realistic pulsatile flow and stretch signaling in cultured vascular cells. *Am J Physiol Cell Physiol* 279:C797-C805.
- Putnam AJ, Cunningham JJ, Dennis RG, Linderman JJ, Money DJ. 1998. Microtubule assembly is regulated by externally applied strain in cultured smooth muscle cells. *J Cell Sci* 111(Pt22):3379-3387.
- Qiu Y, Tarbell JM. 2000. Interaction between wall shear stress and circumferential strain affects endothelial cell biochemical production. *J Vasc Res* 37:147-157.
- Quinn TP, Schlueter M, Soifer SJ, Gutierrez JA. 2002. Cyclic mechanical stretch induces VEGF and FGF-2 expression in pulmonary vascular smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 282(5):L897-L903.
- Raimondi MT, Boschetti F, Falcone L, Fiore GB, Remuzzi A, Marinoni E, Marazzi M, Pietrabissa R. 2002. Mechanobiology of engineered cartilage cultured under a quantified fluid-dynamic environment. *Biomech Model Mechanobiol* 1(1):69-82.
- Resnick N, Yahav H, Shay-Salit A, Shushy M, Schubert S, Zilberman LC, Wofovitz E. 2003. Fluid shear stress and the vascular endothelium: For better and for worse. *Prog Biophys Mol Biol* 81(3):177-199.
- Robling AG, Hinant FM, Burr DB, Turner CH. 2002. Improved bone structure and strength after long-term mechanical loading is greatest if loading is separated into short bouts. *J Bone Miner Res* 17:1545-1554.
- Shreiber DI, Barocas VH, Tranquillo RT. 2003. Temporal variations in cell migration and traction during fibroblast-mediated gel compaction. *Biophys J* 84(6):4102-4114.
- Smalt R, Mitchell FT, Howard RL, Chambers TJ. 1997. Induction of NO and prostaglandin E2 in osteoblasts by wall-shear stress but not mechanical strain. *Am J Physiol* 273(4 Pt 1):E751-E758.
- Tanaka SM, Li J, Duncan RL, Yokota H, Burr DB, Turner CH. 2003. Effects of broad frequency vibration on cultured osteoblasts. *J Biomech* 36(1):73-80.
- Tilles AW, Baskaran H, Roy P, Yarmush ML, Toner M. 2001. Effects of oxygenation and flow on the viability and function of rat hepatocytes cocultured in a microchannel flat-plate bioreactor. *Biotechnol Bioeng* 73(5):379-389.
- Torok E, Pollok JM, Ma PX, Vogel C, Dandri M, Petersen J, Burda MR, Kaufmann PM, Kluth D, Rogiers X. 2001. Hepatic tissue engineering on 3-dimensional biodegradable polymers within a pulsatile flow bioreactor. *Dig Surg* 18(3):196-203.
- Truschel ST, Wang E, Ruiz WG, Leung SM, Rojas R, Lavelle J, Zeidel M, Stoffer D, Apodaca G. 2002. Stretch-regulated exocytosis/endocytosis in bladder umbrella cells. *Mol Biol Cell* 13(3):830-846.
- Tzima E, Del Pozo MA, Kiesses WB, Mohamed SA, Li S, Chien S, Schwartz MA. 2002. Activation of Rac1 by shear stress in endothelial cells mediates both cytoskeletal reorganization and effects on gene expression. *EMBO J* 21(24):6791-6800.
- Uematsu M, Ohara Y, Navas JP, Nishida K, Murphy TJ, Alexander RW, Nerem RM, Harrison DG. 1995. Regulation of endothelial cell nitric oxide synthase mRNA expression by shear stress. *Am J Physiol* 269:C1371-C1378.
- Van der Pauw MT, Klein-Nuland J, van den Bos T, Burger EH, Everts V, Beertsen W. 2000. Response of periodontal ligament fibroblasts and gingival fibroblasts to pulsating fluid flow: Nitric oxide and prostaglandin E2 release and expression of tissue non-specific alkaline phosphatase activity. *J Periodontol Res* 35(6):335-343.
- Vlahakis NE, Hubmayr RD. 2003. Response of alveolar cells to mechanical stress. *Curr Opin Crit Care* 9(1):2-8.
- Wang JH, Goldschmidt-Clermont P, Wille J, Yin FC. 2001. Specificity of endothelial cell reorientation in response to cyclic mechanical stretching. *J Biomech* 34(12):1563-1572.
- Warden SJ, Turner CH. 2004. Mechanotransduction in the cortical bone is most efficient at loading frequencies of 5-10 Hz. *Bone* 34(2):261-270.
- Waters CM, Chang JY, Glucksberg MR, DePaola N, Grotberg JB. 1997. Mechanical forces alter growth factor release by pleural mesothelial cells. *Am J Physiol* 272:L552-L557.
- Wirtz HR, Dobbs LG. 2000. The effects of mechanical forces on lung functions. *Respir Physiol* 119(1):1-17.
- Zhao S, Suci A, Ziegler T, Moore JE, Jr., Burki E, Meister JJ, Brunner HR. 1995. Synergistic effects of fluid shear stress and cyclic circumferential stretch on vascular endothelial cell morphology and cytoskeleton. *Arterioscler Thromb Vasc Biol* 15:1781-1786.