New Fluid Flow System for Simulation of Mechanical Loading to Bone Cells During Human Gait Cycle

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Abstract

Mechanical loading to bone cells using simple sine wave or constant wave fluid flow has been widely used for in vitro experiments. Human gait is characterized by a complex loading to bones of lower extremities which results from a series of events consisting of heel strike, foot flat and push-off during the stance phase of the gait cycle. Telemetric force analyses have shown that human femora are subject to multiphasic loading. Therefore, it would be ideal if the physiologic loading conditions during human walking can be used for in vitro mechanotransduction studies. Here, for a mechanotransduction study, we develop a fluid flow system (FFS) in order to simulate human physiologic mechanicalloading on bone cells. The development methods of the FFS including the COR (Center for Orthopedic Research) monitor program are presented. The FFS could generate various multiphasic loading conditions of human gaits with output flow. Wall shear distribution was very uniform, with 81 % of the effective loading area of the culture on a glass slide. Our results demonstrated that the FFS provide a new translational approach for unveiling molecular mechanotransduction pathways in bone cells.

Key words : mechanotransduction, mechanical loading, wall shear, human gait, bone cells

I. INTRODUCTION

The mechanical loading of bone is a potent stimulus for new bone formation. At the cellular level, osteocytes have been described as the primary mechanosensors within bone. Fluid shear stress in response to mechanical loading is known to trigger the expression of key effectors involved in osteoblastic and/or osteoclastic cellular activity (Turner CH et al., 2005; Tanaka SM et al., 2005). Several studies using histomorphometry and high-resolution computed tomography have shown that mechanical loading or unloading affects the quality of bone tissue and architectural characterization of human lumbar cancellous bone (Fischer KJ et al., 1998; Hsieh YF et al., 2001; Cendre E et al., 1999 Judex S et al., 2003). In order to understand the effects of mechanical loading on bone cells at a cellularand molecular level, various in vitro loading systems have been developed by many investigators to apply strains, unidirectional flow and oscillatory fluid flow (Watanabe S et al., 2005; Farokhzad OC et al., 2005; Alford AI et al., 2003). However, since in vivo mechanical loading of bone generates fluid shear stress, which is thought to be dynamic in nature, oscillatory fluid flow may be a more physiologic fluid flow profile than unidirectional fluid flow. The predicted physiologic rangeof fluid flow shear is reported to be around 5-30 dynes/cm^{2}occurring within bone in vivo (Weinbaum S et al., 1994). Meanwhile, various loading conditions have so far been applied to bone cells at the cellular and molecular level to understand osteogenic response. These loading conditions are 1-20 dynes/cm^2 at 1 Hz (You J et al., 2000 Li YJ et al., 2004), and 1 to 20 dynes/cm^2 at direct current (DC) (Elfervig MK et al., 2001 Kapur S et al., 2005; Genetos DC et al., 2004 Smalt R et al., 1997 Bacabac RG et al., 2005). Based on all the results accumulated so far, it is important to understand the mechanotransduction pathways which may be used to provide a new avenue to enhance human bone health (Bacabac RG et al., 2005; McGarry JG et al., 2005; Norvell SM et al., 2004; Inoue D et al., 2004; Kreke MR et al., 2004; Ponik SM et al., 2004).

For this purpose, simulations of physiologic loading patterns are a prerequisite for the generation of clinically significant data. Although the exact association between flow movement

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in bone and human activities has not been found, mechanical loading profiles caused by human activities have been assumed to be correlated withthe pressure gradients that drive fluid through the porous channels of bone and subsequently inducea similar pattern of intra-osseous interstitial fluid flow based on computational and mathematical models (Judex S et al., 1997; Mi LY et al., 2005). The pressure gradients across the bone determine the fluid movement within human cortical bone (Dillaman RM et al., 1991; Schmidt SM et al., 2005; Qin YX et al., 2003; Knothe Tate ML, 2003 Winet H, 2003).

Therefore, the purpose of this study is to develop a translational fluid flow system (FFS) to simulate human gait-like oscillatory fluid flow in bone. Telemetric in vivo loading data that we used as human gait profiles are based on Bergmann G. et al.'s measurements of hip contact forces causing the pressure gradient across. Their studies have shown that physiologic loading to femora occurs in multi-phasic patterns which change during normal (NW), fast (FW), upstairs (UW), and downstairs walking (DW).

Since human gaits generate the complexity of bone's normal physiological environment and interstitial fluid flow profiles, the FFS will provide an important comparison of the response of commonly used bone cell lines todifferent fluid shear profiles thereby giving new insight into the process of mechanotransduction in bone cells. However, we cannot rule out the possibility that the difference between the actual and predicted physiologic flow profiles may account for differences

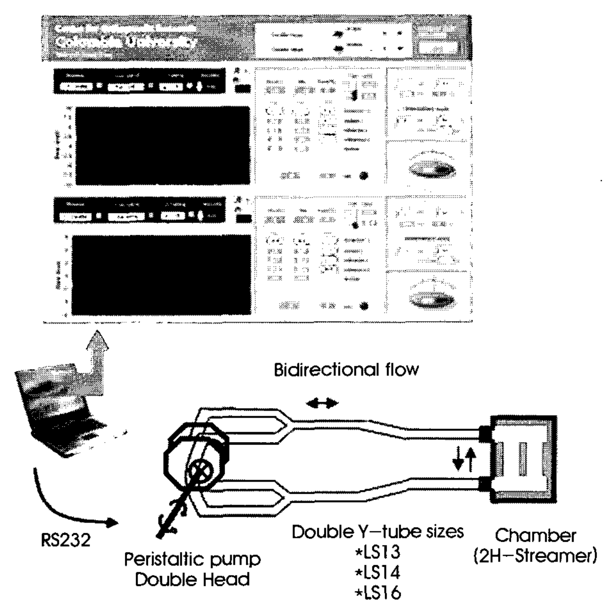

Fig. 1. The components of our hydraulic configuration consist of a chamber, double Y-tube, peristaltic pump, and control computerincluding the COR monitor program.

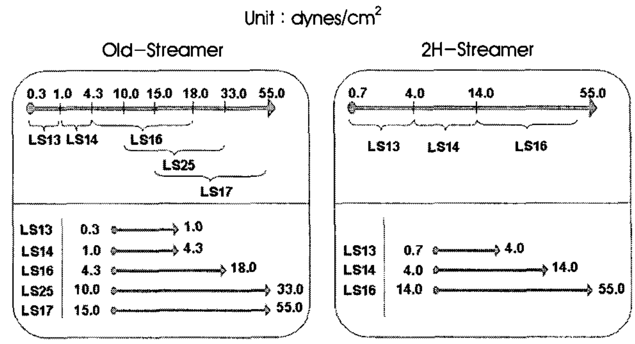

Fig. 2. Y-tube selection guide according to the different levels of the shear stress for the 2H-streamer and old-streamer.

in physiologic loading studies to femora because the properties of physiologic profile cannot be directly measured in bone.

II. MATERIALS AND METHODS

Hydraulic Configuration

The components of our hydraulic configuration consist of a chamber, double Y-tube, peristaltic pump, and control computer as shown in FIGURE 1. The COR software monitor program developed at the Center for Orthopedic Research (COR), Columbia University activates the pump drive. The performance of the parallel flow chamber (2H-streamer) in the context of uniform distribution of shear on the glass slide on a rectangular duct was verified using the FLUENT program. The chamber is made of a transparent acrylic material to monitor hydrodynamic states under fluid flow loading.

For implementation of an optimal hydraulic system, a peristaltic pump (LS brushless computer-programmable drive, Cole-Parmer Instrument Co., Vernon Hills, IL, USA) was used. This peristaltic pump is driven by a motor between a range of 10 RPM to 600 RPM. A pump with a double-head (L/S® Easy-Load II pump head 77201-60, Cole-Parmer Instrument Co., Vernon Hills, USA) consisting of four rollers was assembled with double-Y tubing. Any range (0.7~55.0 dynes/cm2) of in vitro shear stress for the 2H-streamer is adjustable by changing the volumetric flow rate and double-Y tube size (FIGURE 2).

Computational Flow Dynamics (CFD)

For the accurate analysis of CFD, a whole chamber was numerically examined by utilizing the commercially available FLUENT program (Fluent Inc., Lebanon, NH, USA). We calculated the wall shear stress and confirmed its uniform distribution on a glass slide within the 2H-streamer. More than 200,000 meshes were generatedto represent the accurate

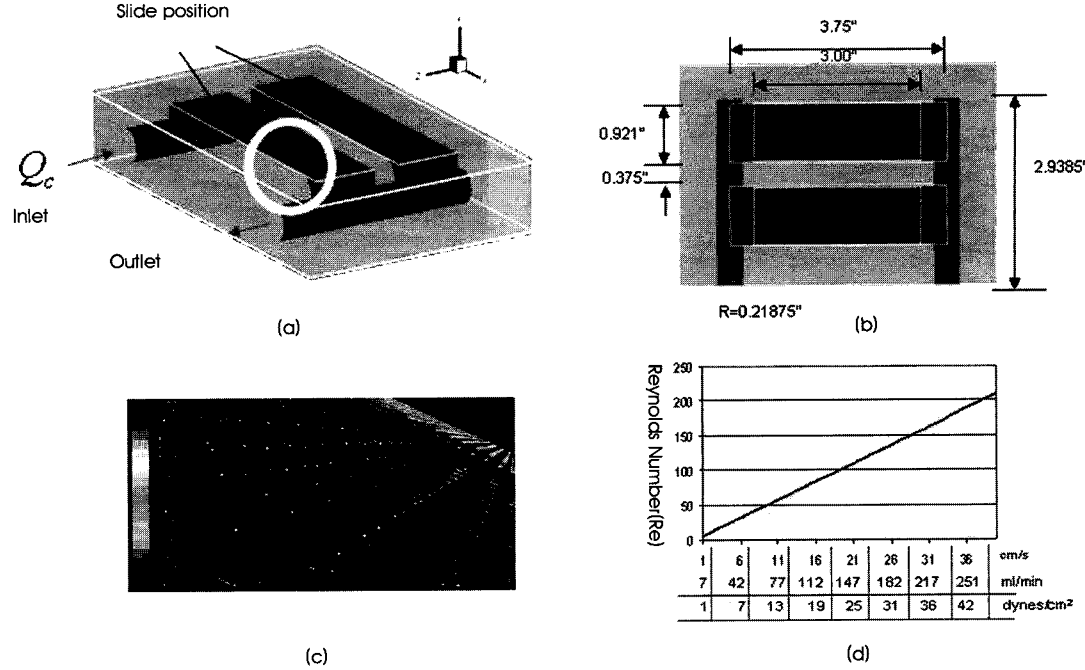

Fig. 3. The wall shear distribution on a rectangular duct and 3D laminar flow were numerically investigated using the FLUENT program; (a) new chamber, (b) the 2H-streamer with the flow area width of 23.393 mm and the flow area height of 0.51 mm, (c) 3D laminar flow enlarged from the circle of (a), and (d) data plot of the Reynolds number (Re) with flow rate, flow velocity, and the wall shear stress in the streamer.

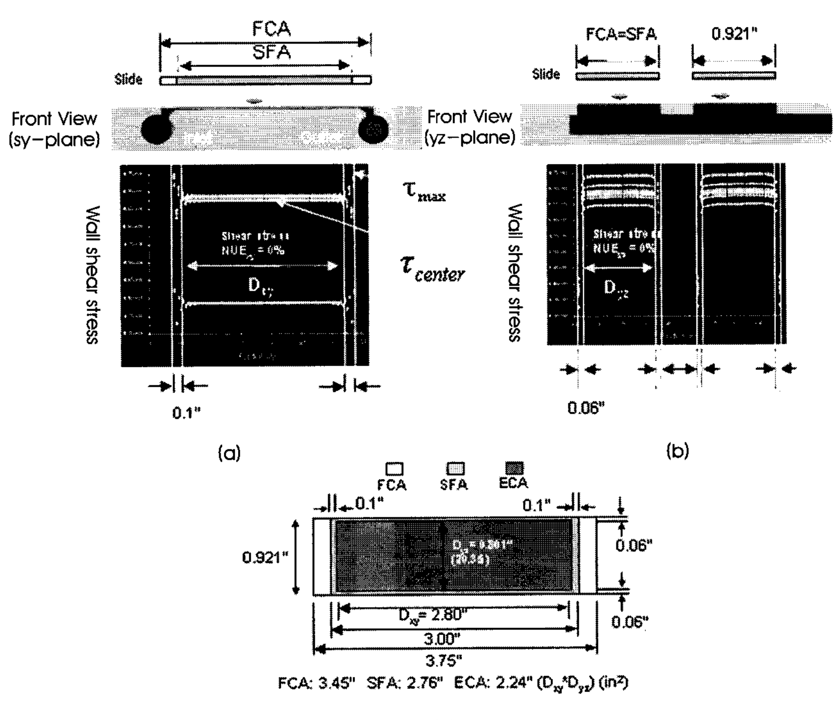

Fig. 4. A fully developed shear flow region (FDSFR) was determined by the region that non-uniformity error (NUE) is 0 % in both xy-plane and yz-plane on a glass slide; (a) NUE = 0 % in xy-plane, (b) NUE = 0 % in yz-plane, and (c) the Effective Culture Area (ECA) was calculated from the Dxy and Dyz. FCA stands for the flow contact area, and SFA the slide flow area.

dimension of the 2H-streamer chamber in FIGURE 3(a) and FIGURE 3(b). The whole wall shear distribution and 3D laminar flow were calculated as shown in FIGURE 3(c). Actual performance of the 2H-streamer (APS, %) was defined as the uniform area of its wall shear stress. To define the uniformity of wall shear distribution in the 2H-streamer, a fully developed shear flow region (FDSFR) was determined by the non-uniformity error (NUE, %) of the following equation.

$$NE_{xy\,\mathrm{or}\,zy}(\%) = \left.\frac{(\tau_{center} - \tau_{\max})}{\tau_{center}}\right|_{SFA} \times 100 \qquad (1)$$

NUE was defined as the normalized difference between the maximum gradient value of shear ($\tau_{\max}$) and any ideal center value (τ_{center}) of shear in the y-axis parallel to the glass slide flow area (SFA). FIGURE 4(a) and 4(b) showed the region of NUE = 0% in both the xy-plane and the yz-plane for determination of the FDSFR, respectively. The FDSFR corresponded to the total region where NUE representing the product of D_{xy} and D_{yz} is zero. An effective area within the SFA where NUE is 0 % wasdefined as an effective culture area (ECA). As a result, we could calculate the APS in terms of ECA and SFA with the following equation. Therefore, the APS was defined as the ratio of ECA to SFA. We obtain each APS of 81.2 % in two rectangular ducts as shown in FIGURE 4(c).

$$APS(\%) = \frac{ECA}{SFA} \times 100 \qquad (2)$$

Under the physiological shear loading caused by interstitial fluid flow in bone, the shear stress falls in the range of a maximum 24 dynes/cm2 based on studies using high-energy synchrotron X-ray diffraction (Almer JD et al., 2005) and stereophotogrammetry (Ateshian GA et al., 1991). It is essential to characterize the laminar flow in the 2H-streamer in terms of the Reynolds number (Re). In general, a Reynolds number less than 1600 indicates that the flow profile is

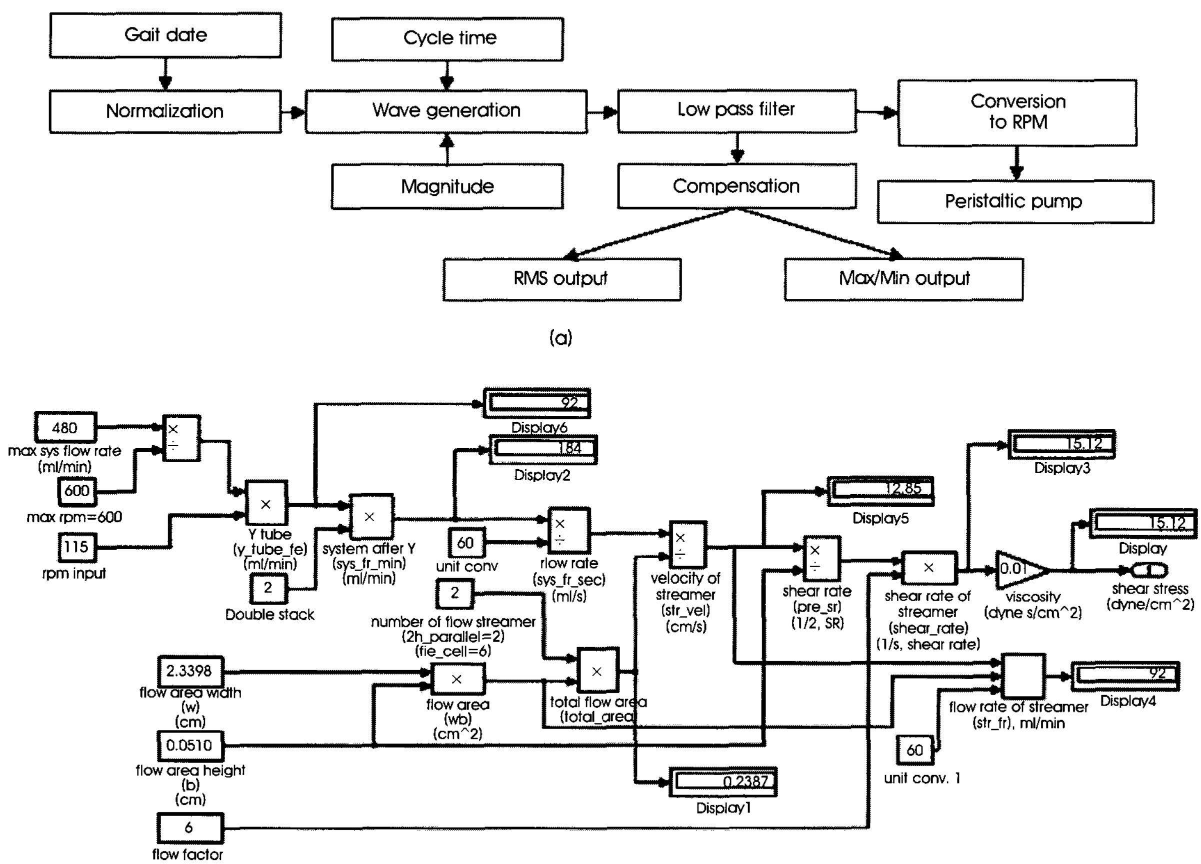

Fig. 5. The main algorithms for the COR monitor program are shown:(a) the algorithms covering from the data collection to the generation of human gait loading conditions, and (b) the relationship among shear stress, fluid viscosity, dimension of a streamer, Y-tube size, and motor RPM.

laminar and it is linear with respect to the flow rate.

$$Re = \frac{\rho V h}{\mu} \tag{3}$$

ρ is the fluid density, V the average fluid velocity inside the 2H-streamer, h the 2H-streamer height, and μ the fluid viscosity. A plot of the Reynolds number with flow rate and wall shear stress in the 2H-streamer witha nominal height of 0.510 mm and a width of 23.398 mm is shown in FIGURE 3(d).

COR monitor Program

The COR software monitor program was newly developed in order to simulate various mechanical loading conditions of human gaits using the LABVIEW environment (National Instruments Inc., Austin, TX, USA). FIGURE 1 shows a screen capture of the COR monitor program. FIGURE 5(a) shows a block diagram of algorithms covering from the data collection to the generation of human gait loading conditions. The normalization of human gaits was performed to control a shear stress on the COR monitor program easily. The magnitude of a shear stress resulted in the normalized value times arbitrary value. A low pass filter (LPF) with a cutoff frequency of 5 Hz, then, was used to generate a smooth waveform to represent a closer physiologic viscous fluid environment of bone. Gait analysis on four different patterns by FFT allowed for the introduction of that cutoff frequency as shown in FIGURE 6, although frequencies higher than 5 Hz showed very small magnitudes in other harmonics. The shear stress in the hydraulic configuration was calculated in terms of motor RPM and tubing size in the MATLAB Simulink program(Mathworks Inc, Natick, MA, USA) (FIGURE 5(b)).

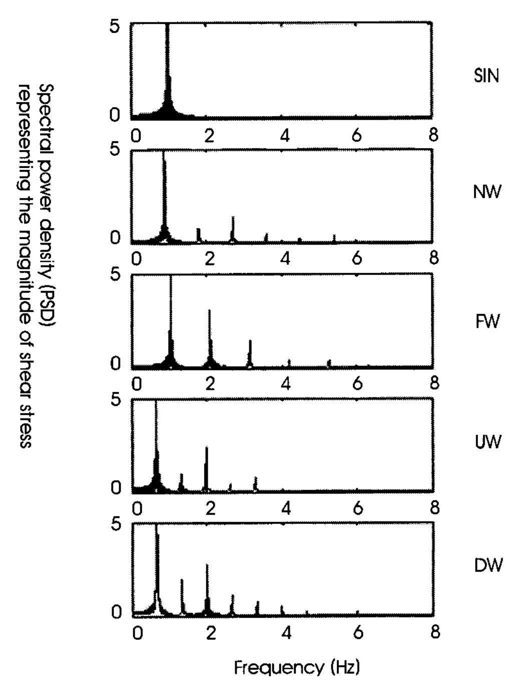

Fig. 6. Spectral analysis of human gaits based on the FFT was performed. Four human gait patterns had many different components within approximately 10 Hz. NW, FW, UW, and DW stand for normal walking, fast walking, upstair walking, and downstair walking, respectively.

The COR monitor program also accepted different types of streamers, different sizes of circular tubes depending on flow rate, and various human walking patterns including sine wave, pulsatile wave, and constant wave. For the generation of intermittent modes, a resting state called zero shear stress could be inserted between waves. Estimates of shear stress, flow velocity, and flow rate in the streamer and the circular tube were displayed in real time with maximum/minimum and root mean square (RMS) values. The RMS value represented the effective value of a time-course flow which gave the equivalent steady effect. The RMS equation was programmed in the COR monitor program.

$$Q_x = \sqrt{\frac{1}{n}\sum_{i=0}^{n-1} x_i^2} \tag{4}$$

Q_x is the RMS value, and n is the number of elements in x.

Hydraulic Performance

Hydraulic performance was evaluated in terms of the system RMS flow rate, cycle time, and duty cycle(%) representing the portion of an on-state time in one period of the intermittent mode. To measure an accurate flow rate (ml/min) while the peristaltic pump was operated by the COR monitor program, an experimental flow volume-based measurement was used. This measured the amount of the fluid volume in each different size of the circular tubes using the cylindrical glass bottle with a vertical ruler in ml units.

Multiphase Human Walking Analysis

Human gaits including fast walking (FW), normal walking (NW), downstair walking (DW), and upstair walking (UW) were collected from the data measured in hip contact (Bergmann G et al., 2001). These data were collected from measurements of the hip contact forces of four patients using an implanted telemetric device on the surface of the proximal femora. Such human gait patterns for the prediction of bone interstitial fluid flow frequency components were analyzed by utilizing the Fast Fourier Transform (FFT) technique, converting decomposed signals into sine and cosine functions of different frequencies The majority of the spectrum energy for four collective gait

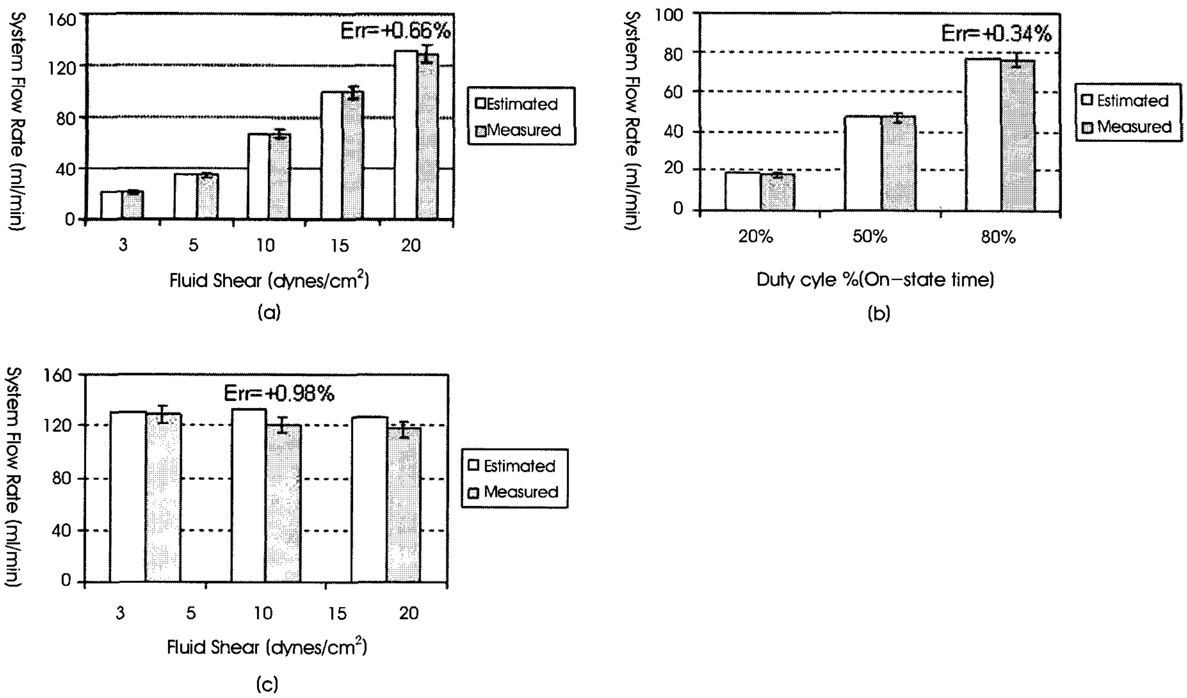

Fig. 7. Hydraulic flow rate (RMS, ml/min) of 1 Hz sine wave was measured. (a) The results showed almost no the difference between estimated and measured flow rates in 3, 5, 10, 15, and 20 dynes/cm2 (b) the flow rates according to 3 steps of 20 %, 50 %, and 80 % duty cycle in a specific period of 10 sec in the intermittent mode were evaluated; (c) at the higher shear stress of 20 dynes/cm2 and a frequency of 2 Hz, maximum difference was +9 %. (Y error bar of 5 %)

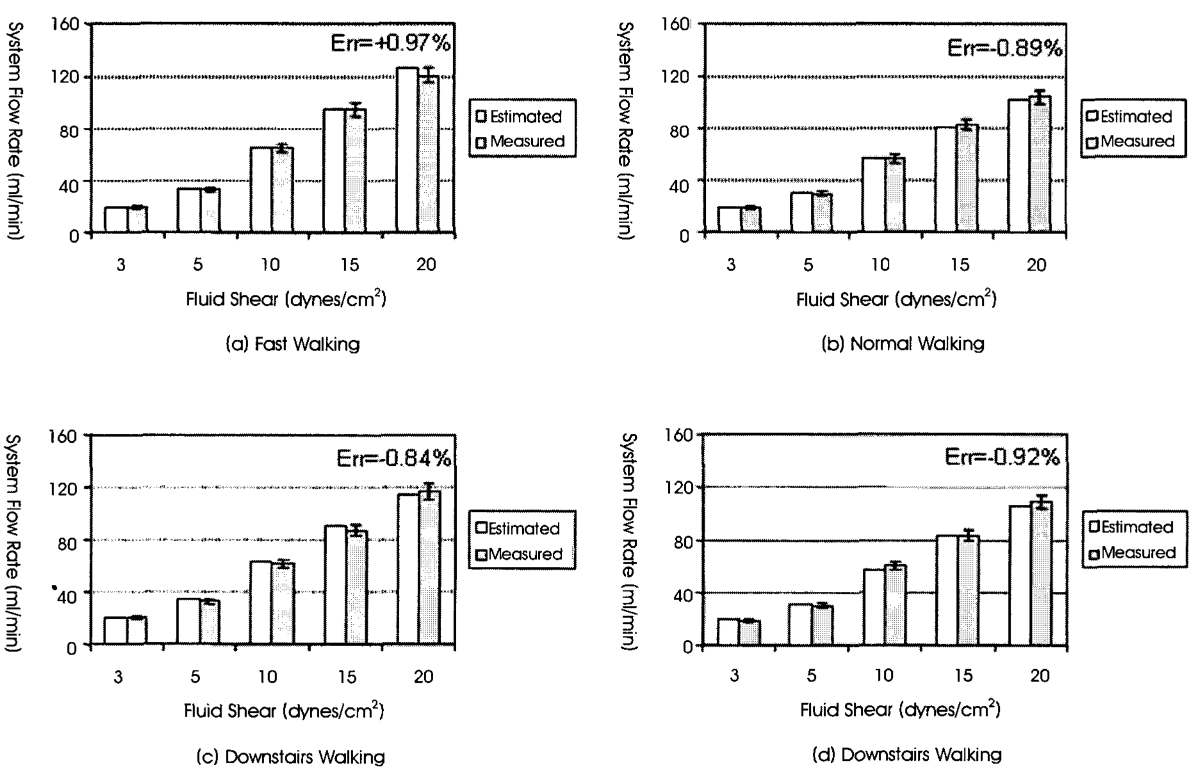

Fig. 8. Hydraulic performance of flow rate reflecting the physiologic mechanicalloadings to the femur during the human gait cycle showed a little difference between calculated and measured outflow rates: (a) fast gait, (b) normal gait, (c) downstair gait, and (d) upstair gait. (Y effort bar of 5 %)

patterns had many different frequency components within approximately 10 Hz as shown in FIGURE 6.

Pump-Generated Human Gaits

The pump-generated hydraulic human gaits were confirmed by evaluating the similarity between the input waveform and output waveform during pump operation. For an output waveform, the fluid pressure driven by the pump was measured inside the tubing. For measurement of this fluid pressure, the bidirectional relative pressure sensor (PX186-015BD5V, OMEGA Engineering Inc, Stamford, Connecticut, USA) was equipped with an extra tube bifurcated with the main circular tubein the fluid closed-loop hydraulic system. The analog sensor output was transferred to PC from an Analog-to Digital Converter (NI USB-9161, National Instruments Inc., Austin, TX, USA) via USB port to store digital data. The input waveform consisted of human gait signals already designed in the COR monitor program. A high speed pump (Masterflex® L/S computer-compatible pump, Cole-Parmer Instrument Co., Vernon Hills, IL, USA) and adouble-head (L/S®Easy-Load II pump head 77201-60, Cole-Parmer Instrument Co., Vernon Hills, USA) were equipped with Y-connector tubing to develop low pulsation flow output.

II. RESULTS

Hydraulic Performance

Hydraulic flow rate (RMS, ml/min) of 1 Hz sine wave was measured more than at least twice at shear levels of 3, 5, 10, 15, and 20 dynes/cm2. The results showed no significant difference between estimated flow rates and measured ones in specific ranges as shown in FIGURE 7(a). The flow rate error at 20 dynes/cm2, where maximum deviation occurred, was slightly overestimated by+1.53%. For the evaluation of an intermittent mode with differing on-state time (or duty cycle) at a period of 10 sec, flow rates according to three duty cycles of 20 %, 50 %, and 80 % were measured in FIGURE 7(b). In this case, 1 Hz sine waves with a maximum and minimum of 15 and 0 dyne/cm2 were applied to make a unidirectional flow output. The maximum error between estimated and measured values ranged from +0.98 % to -0.66 %. At higher shear stress (20 dynes/cm2) with a peak frequency of 2 Hz, the difference was +9 % in FIGURE 7(c). In hydraulic evaluation of the flow rate reflectingphysiologic mechanical loadings to the femur during the human gait cycle, there was little difference between calculatedand measured outflow rates at various shear strengths as shown in FIGURE 8. In particular, fast (FIGURE 8(a)) and normal (FIGURE 8(b)) gaits exhibited the highest

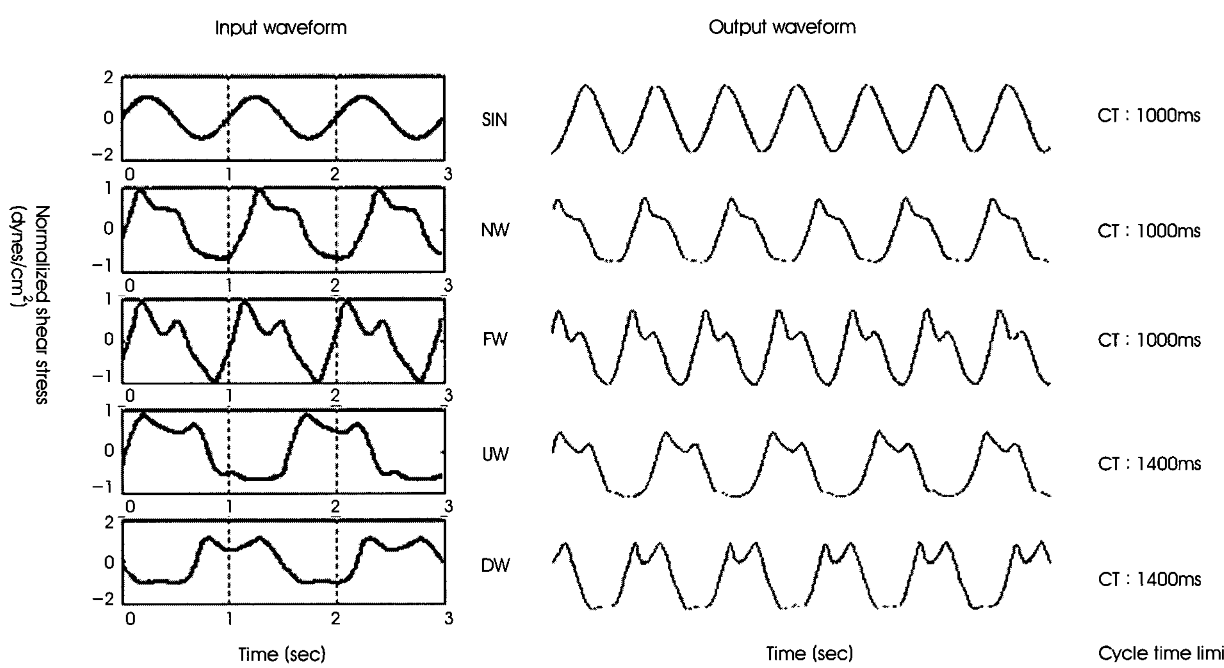

Fig. 9. A close similarity between input waveform and output waveform measured by pressure sensor was obtained. The cross correlation of two waveforms for sine wave, NW, FW, UW, and DW was calculated as r=0.99, r=0.96, r=0.95, r=0.97, and r=0.98, respectively. CT is cycle time. NW, FW, UW, and DW stand for normal walking, fast walking, upstair walking, and downstair walking, respectively.

Table 1. Theoptimal ranges for mechanical signal cycles and Y-tubes for flow capacity were shown.

2H-Streamer in a Double Head — Unit : dynes/cm^2(shear)

	Shear Range	RPM Range	Mechanical Signals	Cycling Time Limit	Y-tube
LS/13	0.7~4	102~406			
LS/14	0.7~4	113~422	Shine Wave	>800ms (1.25Hz)	
LS/16	0.7~4	115~419	Fast Gait	>800ms (1.25Hz)	
Old-Streamer in a Double Head			Normal Gait	>1000ms (1 Hz)	
	Shear Range	RPM Range	Downstair Gait	>1400ms (0.71Hz)	LS/13 LS/14 LS/16 LS/17
LS/14	1.0~4.3	102~422	Upstair Gait	>1400ms (0.71Hz)	
LS/16	4.3~18	112~411	Pulse wave	>1000ms (1 Hz)	
LS/17	15~55	116~419	Dc	No limit	

accordance when the normal and fast gaits were generated at 1,000 ms, and downstair and upstair gaits at 1,400 ms.

In vitro Pump-Generated Fluid Flow Simulating the Mechanical Loading to the Femur During Human Gait Cycle

A close similarity between the input waveform and output waveform was shown in FIGURE 9. Output waveform of the fluid pressure inside LS/13 tubing(Lab. Standard tubing (LS/13®, inner diameter with 0.8 mm), Cole-Parmer Instrument Co., Vernon Hills, USA) generated by the pump was measured using a bidirectional relative pressure sensor. Pure sinusoidal waves and fast gait, and pulse wave and normal gait required a cycle time limit of approximately 800 ms (1.25 Hz) and 1000 ms (1 Hz) to generate an accurate outflow, respectively. Downstair and upstair gaits that had comparatively high frequency components in the spectral analysis required a cycle time limit of 1400 ms (0.71 Hz). The optimal ranges for mechanical signal cycles and Y-tubes for flow capacity that determined the maximum shear magnitude were obtained by conducting experiments as shown in TABLE 1. The shear ranges were divided into three groups according to differences in sizes of the inner diameter (ID) of the Y-tube. The shear ranges of the first group, which uses LS/13 with 0.8 mm ID, ranged from 0.7 to 4 dynes/cm2. The shear ranges of the second group with 1.6 mm ID and the third group with 3.1 mm ID ranged from 4 to 14 dynes/cm2, and from 14 to 55 dynes/cm2, respectively. The distinction among these groups was determined based on the motor RPM in order to obtain accurate hydraulic performance with low pulsation flow.

Ⅲ. DISCUSSION

The goal of this study was to develop the translational fluid flow system (FFS) in order to compare the effects of human gait-like oscillatory fluid flow profiles on important mechanotransduction pathways. Although these fluid flow profiles are inherently different in certain parts of the body, the FFS for delivering human gait fluid flow in femora was set up to have as many similarities as possible. We believe that the FFS can be a simple and technically advanced device for in vitro mechanical transduction studies in both osteoblasts and osteocytes (FIGURE 1). The COR monitor program could simulate various human gait patternsincluding sine wave, pulsatile wave, and constant wave in the new hydraulic configuration. Highly predictable correlation between input signal and pump-generated output signal was successfully verified (FIGURE 9). The magnitude and frequency of human gait-like fluid shear profiles could be controlled on the COR monitor program. The main differences between conventional sine wave and human gait profile were the characteristics of frequency the bone cells were exposed to. A human gait profile with a 1 Hz normal walking gait had wide frequency ranges from 0.2 Hz to 10 Hz, while sine wave had a distinct frequency of 1 Hz. Adaptive responses of bone cells were more sensitive to high frequency (above 4 Hz) and low magnitude loads (Bacabac RG et al., 2005).

The new parallel chamber designed from the results of the FLUENT simulations exhibited a high uniformity of wall shear distribution higher than 81 % effective flow area on a glass cell culture slide (FIGURE 4). Other advantages were as follows: (1) minimization of cell culture medium with approximately 20 ~ 30 ml depending on the Y-tube size, (2) complete description of the relationships among input parameters (maximum system flow rate, medium viscosity), and dimension of a rectangular duct, and output parameters (shear stress, flow velocity, and flow rate) (FIGURE 5(b)), (3) mathematical equations to define the APS of a chamber (Equations 1 and 2), and (4) an intermittent loading mode capable of inserting an arbitrary duration of off-time in continuous mode for any waves. Additionally, all components of the hydraulic configuration and their set-up have been optimizedto minimize the introduction of contaminants.

However, the program execution time of every 250 ms by a serial communication protocol was too long to generate a fast cycle. Cycling times ofmore than 800 ms for sine wave and fast gait, 1000 ms for normal gait and pulse wave, and 1400 ms for downstair and upstair gait was required(TABLE 1). Optimization of the execution time by changing the motor RPM and a serial communication speed should be incorporated into future designs. Our assumption that human gaits mightcause alterations in the bone interstitial fluid flow was based on several studies by other investigators (Dillaman RM et al., 1991; Schmidt SM et al., 2005; Qin YX et al., 2003; Knothe Tate ML, 2003).

In conclusion, we believe that our FFS is very useful for the in vitro mechanotransduction studies focusing on the effect of human gait on bone cells of the lower extremities. In addition, the whole development processes presented will provide key information to aid in optimizing hydraulic configurations, chambers, and software programs, but gait-induced physiologic loading profiles should be further interpreted and discussed through a computational analysis of bone matrix.

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