MICROFLUIDIC FLUID SHEAR DELIVERY SYSTEM FOR IN VITRO 
BONE MECHANOREGULATION
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ABSTRACT 
We present a pneumatically-driven microfluidic actuation device for delivering oscillatory fluid flow to bone cells in culture. In this two-layer system, the bottom layer contains fluidic channels and miniature cell culture chambers. The top layer consists of a pneumatically-actuated channel that subjects cells to physiological levels of fluid shear. We fabricated and tested this device using particle image velocimetry and validated that it provides physiological levels of shear stress. This device will facilitate high-throughput assays where bone cells are cultured under similar mechanical conditions to their native in vivo environment, which will allow us to better understand mechanical-disuse induced osteoporosis.

KEYWORDS: Pneumatic actuation, Fluid shear, Cell biomechanics, Bone mechanoregulation

INTRODUCTION
Decreased mechanical loading of bone due to prolonged bed-rest or microgravity causes disuse osteoporosis. Bone is a dynamic tissue that adapts to changes in mechanical loading, which has been shown to induce oscillatory fluid flow in the bone microporosity. Osteocytes (cells embedded in bone matrix) are believed to be responsible for detecting changes in mechanical loading of bone and, in response, regulating bone remodeling. However, the cellular mechanisms are not fully understood largely due to the lack of physiologically meaningful cell culture models that deliver mechanical stimuli in vitro. Due to the length of individual experiments and the large size of the parameter space to be explored, compact, high-throughput systems capable of performing multiple experiments in parallel are of the essence.

Commonly, mechanical forcing is achieved externally using syringe pumps. While different types of fluid profiles can be generated, the elasticity of microfluidic lines causes an unwanted time lag and alteration of the time characteristics of fluid velocity and shear stress profiles. Parallel plate flow chambers have been widely used for subjecting bone cells to physiological levels of fluid shear [1] but have limited scalability and ability to controllably screen frequencies of up to 60Hz. A more accurate approach for reducing time lag and producing well-defined levels of shear stress is to employ local mechanical forcing on a microfluidic chip on which cells are cultured and subjected to physiological levels of fluid shear.

EXPERIMENTAL
We present a pneumatically-actuated approach to delivering physiological oscillatory fluid flow to cells in vitro. Pneumatic actuation has been used in various microfluidic applications for valves, flow regulation and bubble removal [2-4]. In our two-layer system, the bottom layer contains fluidic channels and a cell culture chamber (700μm wide, 12mm long, 50μm deep), and the top layer consists of a pneumatic actuation channel (900μm wide, 11.9mm long, 70μm deep). We apply vacuum to the pneumatic layer of the microdevice such that the PDMS membrane of the underlying fluidic channel is deflected up. Membrane deflection causes fluid in the bottom microchannels to be drawn back, while during rest phase (when atmospheric pressure is returned) the PDMS membrane returns to its original position and the underlying fluid is pushed forward (Figure 1). Periodic changes of pressure in the actuation channel, therefore, establish the desired oscillatory flow shear conditions in the bottom channel and particularly the cell culture area.

Figure 1: A) Cross section of the actuation area. As vacuum is turned on (B) and off (C), a thin PDMS membrane is deflected up and down, thus inducing oscillatory fluid motion in the underlying microfluidic channel.

To quantify fluid shear stress in the microdevice, microchannels were filled with a 1% aqueous solution of 1μm diameter fluorescent polystyrene particles and fluid velocity was measured using microscale particle image velocimetry (PIV), which produced an array of velocity vectors over time.
RESULTS AND DISCUSSION

Data analysis of velocity vectors obtained using PIV showed a temporal evolution of the mean velocity describing quasi-oscillatory fluid flow at a frequency of 1 Hz within the liquid-filled microchannel, as expected. By changing the overlap area between the pneumatic and fluidic channels, we were able to obtain different shear stress levels, as indicated in Figure 3. We tested large (12.8mm²), medium (8.9mm²) and small (4.4mm²) overlap areas.

We also determined shear levels for various actuation frequencies (1, 2, 5, 10Hz) and found similar quasi-harmonic flow profiles, as shown in Figure 2 for a medium overlap device.

We demonstrate the scalability of this technique by designing a multi-well system which would simultaneously subject 32 cell culture areas to eight different shear conditions: two actuation frequencies and four shear stresses (Figure 5). This device is compatible with the footprint of a 96-well plate, thus providing us with one third of a well-plate (32 wells) on a single 2x3” device, making this device compatible with conventional cell culture methodologies. This concept may ultimately enable routine screens in high-throughput assays under well-defined levels of shear stress.
Figure 5: A) Pneumatic actuation device adapted to 1/3 of a 96-well plate, delivering 8 shear conditions (4 shear stresses at 2 actuation frequencies). B) Each 9mm x 9mm square is comprised of an outlet well at the top layer, middle pneumatic actuation layer and bottom fluidic layer that contains a cell culture area. C) Multi-well device is compatible with standard multi-channel pipets used in conventional cell culture assays.

CONCLUSION
Our system was demonstrated to deliver quasi-harmonic oscillatory flow profile frequencies and shear stress magnitudes comparable to physiological conditions and conventional (non-microfluidic) flow-loading experiments. The presented on-chip fluid displacement approach provides a useful tool for delivering physiological levels of fluid shear and studying the role of mechanical stimuli on bone cell interaction in vitro. The solution can be easily adapted to other biological scenarios involving mechanical stimulation as in the case of endothelial cells lining blood vessels that are constantly subjected to the pulsating flow of blood. This approach eliminates the need of syringe pumps and other external fluid loading devices, thereby yielding miniature devices that can potentially be mass-produced at low cost and are readily compatible with conventional cell culture equipment (e.g., incubators) and microscopes.

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REFERENCES

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